To the
German Patent and Trademark Office

Munich

Dachau, January 27th 2003

Re Utility Model N° DE 201 16 428 U1
Pharmaceutical compound consisting of amlodipine maleate
Cancellation proceedings
Cancellation petitioner:
CIMEX DEVELOPMENT AG
Hauptstraße 67
CH-4102 Binningen/Basle
Switzerland
Cancellation opponent:
BioOrganics BV
Toernooiveld 134
Nijmegen, Netherlands
My ref.: GL 7 922

Utility Model Cancellation Application

In the matter

CIMEX DEVELOPMENT AG
legally represented by its Board members
Eduard Kny and Jean Lüchinger
Hauptstraße 67, CH-4102 Binningen/Basle, Switzerland

Cancellation Petitioner

Legal counsel: Dr. Stephan G Beszédes, Münchener Straße 80a
85221 Dachau

Authorised recipient

versus

BioOrganics BV, Toornooiveld 134,
legally represented by its manager
6525 EC Nijmegen, Netherlands

Cancellation Opponent

Domestic representative: Maiwald Patentanwälte-GmbH, Ballindamm 37, 20095 Hamburg, Federal Republic of Germany

for the cancellation of Utility Model 201 16 428 U1, the following petitions have been lodged in the name and on behalf of the Cancellation Petitioner:

I. The German Utility Model 201 16 428 U1 be fully cancelled.

II. The Cancellation Opponent be ordered to bear the costs of the cancellation proceedings.

REASONING

The object of Patent Claim 1 for Utility Model 201 16 428 U1 is

A pharmaceutical compound, consisting of an effective volume of amlodipine maleate and at least one pharmaceutically acceptable adjuvans, whereby the compound has a pH between 5.5 and 7.0.

Copies of Utility Model document DE 201 16 428 U1, the utility model application, are submitted as

Annex ASt A

II)

Absence of patentability of the object of Patent Claim 1

On the basis of § 15, para. 1 n° 1 Utility Model Act
In connection with §§ 1 to 3 Utility Model Act

The object of Patent Claim 1 for the German Utility Model 201 16 428 has been anticipated to the detriment of its novelty in the following prior publications.

1) **US Pat nt Specification 5, 155, 120 a copy of which is appended as**

Annex ASt 1

In particular column 2 lines 43 to 45

> "For purposes of oral administration, tablets containing various excipients, such as sodium citrate, calcium carbonate and dicalcium phosphate may be employed"

in connection with column 2 line 18, where the maleate is cited as the amlodipine salt.

According to German Utility Model Specification 201 16 428, in particular page 7, penultimate para, primarily lines 4 to 7

> "logically the use of adjuvans that are pH-inert, i.e. which have little or no effect on the pH value, generally leads to a non-alkali pharmaceutical compound, since the amlodipine maleate mainly acts as its own standardising agent."

pH-intert adjuvans lead to the pH values cited in the German Utility Model Specification 201 16 428, whereby page 7 column 4 of German Utility Model Specification 201 16 428 mentions calcium hydrogen phosphate = dicalcium phosphate as a thinner, i.e. inert adjuvans, in full agreement with US Patent Specification 5, 155, 120 (Annex ASt 1), column 2 line 45, where, in line 46 and also page 7, penultimate para., lines 7 to 9 of German Utility Model Specification 201 16 428

microcrystalline cellulose is cited.

Based on US Patent Specification 5, 155, 120 (Annex ASt 1), in other words, the object of Patent Claim 1 of German Utility Model Specification 201 16 428 is not new.

2) **Specification WO 95/34299, appended as**

Annex ASt 2

in particular page 6, lines 26 to 32

> "Without limiting the invention to the specific compounds listed, the following is a list of representative L-type channel blockers useful in this invention: amlodipine ..... and pharmacologically acceptable salts thereof."

and page 7, lines 27 to 32:

> "Pharmaceutically acceptable salts of L-type calcium channel blockers include the conventional, non-toxic salts from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from .... organic acids such as ....., maleic....."

in connection with page 9 line 25

> "The pH of the solution preferably is between about 6.5 and about 8.0...".

The last place cites a pH range of between ca. 6.5 to 8 for the compound, of which the part of ca. 6.5 to 8.0 falls under the definition of Patent Claim 1 in the German Utility Model Specification 201 16 428.

Also, on the basis of Specification WO 95/34299, the object of Patent Claim 1 of German Utility Model Specification 201 16 428 is not new.

3) **US Patent Specification 6.057.344, copies of which are appended as**

Annex ASt 5

cites mixtures of microcrystalline cellulose (column 11, lines 39 to 44)

> "Usual pharmaceutical media include, for example ..... carriers such as ..... microcrystalline cellulose,....."

with amlodipine maleate (column 10, lines 57 to 61)

> "The pharmaceutical compositions of the present invention comprise (-) amlodipine as active ingredient or a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier and optionally other therapeutic ingredients"

in connection with column 10 line 65 to column 11 line 4

> "Since the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptable non-toxic acids, including inorganic and organic acids. Such acids include ..... maleic."

Page 7, penultimate para, lines 8 to 9 of German Utility Model Specification 201 16 428 "shows" "a compound comprising amlodipine maleate and microcrystalline cellulose" "with generally a pH value of ca. 6". It is precisely this that is involved in US Patent Specification 6.057.344 (Annex ASt 5) (whereby the only carriers cited, alongside microcrystalline cellulose, are starches and sugars, also pH-neutral.

The facts of the case are also unaltered by the fact that US Patent Specification 6.057.344 (Annex ASt 5) concerns optically active (-) amlodipine since German Utility Model Specification 201 16 428, on page 5 para. 3, expressly includes every form of amlodipine maleate and thus also optically active forms (even when these are not cited *expressis verbis*). Moreover in would not represent any inventive activity to transfer the familiar elements from US Patent Specification 6.057.344 (Annex ASt 5) for the (-) amlodipine maleate to the racemic amlodipine maleate since the racemic amlodipine maleate contains half the (-) amlodipine maleate.

II

Moreover the object of Patent Claim 1 of German Utility Model Specification 201 16 428 lacks the inventive element due to the following prior publications.

4) **US Patent Specification 4.590.195, a copy of which is appended as**

Annex ASt 3

in particular Formula I (column 1, lines 37 to 44) and column 2, line 23 with the express mention of the maleate as the salt of the compounds highly similar to amlodipine in connection with column 6, lines 13 to 15

> "For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose,...."

and Examples 62 and 63 with dicalcium phosphate or microcrystalline cellulose as the adjuvans in agreement with German Utility Model Specification 201 16 428, page 7, penultimate para., lines 7 to 9 where it states that a compound consisting of amlodipine maleate and microcrystalline cellulose in general has a pH value of 6 and page 7, penultimate para., lines 4 to 7, where it states that the use of adjuvans that are pH-inert, in general lead to a on-alkaline pharmaceutical composition, and Examples 1 and 2. It is true that the compounds in US Patent Specification 4.590.195 (Annex ASt 3) are a bit different to amlodipine (deviating on the basis of the definition of R^4 in US Patent Specification 4.590.195 (Annex ASt 3), which cannot be hydrogen), however German Utility Model 201 16 428 lacks inventiveness, since the structure concerned involves very similar compounds.

5) **US Patent Specification 4.879.303, copies of which are appended as**

Annex ASt 4

In particular column 1, lines 43 to 50

> "The invention further provides a tablet formulation comprising the besylate salt of amlodipine in admixture with excipients. A preferred formulation includes the besylate salt, a compression aid such as microcrystalline cellulose, an additive to provide sheen to the tablet such as anhydrous dibasic calcium phosphate, a disintegrant such as sodium starch glycollate and a lubricant such as magnesium *(illegible)*

column 3, lines 38 to 39

> "Microcrystalline cellulose is a commonly used compression aid"

and line 4, lines 59 to 63

> "Amlodipine besylate was blended with sodium starch glycollate and anhydrous dibasic phosphate for 5 minutes. This mixture was then sieved, re-blended and sieved again followed by blending with microcrystalline cellulose."

It is true that amlodipine besylate is stressed there as the amlodipine salt however the publication of the formulation with microcrystalline cellulose or dibasic calcium phosphate = calcium hydrogen phosphate however includes amlodipine salts, such as amlodipine maleate (Table 1 in column 2, line 43) or may be interpreted as such. In any event, as a result, the object of Patent Claim 1 of German Utility Model Specification 201 16 428 lacks the inventive element.

Reference should also be made again to Table 1 in US Patent Specification 4.879.303 (Annex ASt 4), where column 2 line 43 cites, for amlodipine maleate is a saturated solution, a pH value of 4.8. This agrees with page 7 penultimate para. of German Utility Model Specification 201 16 428 where it also states that, for this reason, for example, an amlodipine maleate and microcrystalline cellulose composition generally has a pH value of ca. 6. Thus US Patent Specification 4.879.303 (Annex ASt 4),

Table 1 also shows that an amlodipine maleate and microcrystalline cellulose composition has a pH value of ca. 6. Such a composition is, however, cited in column 1 lines 45 to 47, even if this particularly deals with the besylate salt of amlodipine.

6) US Patent Specification 6.057.344 (Annex ASt 5)

Example 8 with the pH-neutral adjuvans lactose and starch, in the latter the amlodipine base was, however, used, in connection with column 10, lines 57 to 61 and column 10 line 65 to column 11 line 4, with the details of the pharmaceutically acceptable salts, and in particular also the maleate as the alternative to the amlodipine base, provides, as a matter of course for the specialist, for the exchange of the amlodipine base in Example 8. This also demonstrates, apart from the absence of novelty as shown in I 3) for the object of Patent Claim 1 of German Utility Model Specification 201 16 428 compared to US Patent Specification 6.057.344 (Annex ASt 5), the absence of the inventive element.

The above clearly shows that the object of Patent Claim 1 of German Utility Model Specification 201 16 428 is not new in relation to established technology and furthermore does not involve any inventive activity.

III

Claims

The claims relating back to Patent Claim 1 of German Utility Model 201 16 428, i.e. the genuine

ones, have no inventive component in relation to the object of Patent Claim 1, already demonstrated as not being new, whereby the majority of the same is not new in comparison to established technology

7) **Patent Claim 2**

a) The pH value cited in Specification WO 95/34299 (Annex ASt 2) on page 9, line 25 of between ca. 6.5 and 8.0 contains the sub-range of ca. 6.5 to 7.0 of the pH range of between ca. 6.0 and 7.0 cited in Patent Claim 2 of German Utility Model Specification 201 16 428.

Thus the object of Patent Claim 2 of German Utility Model Specification 201 16 428 is also not new.

b) Moreover US Patent Specifications 5.155.120 (Annex ASt 1) and 6.057.344 (Annex ASt 5) concern German Utility Model Specification 201 16 428, as can be seen from the statements in I 1) and 3).

c) The German Utility Model Specification 201 16 428 page 7 penultimate para, lines 8 to 9 show, as already stated, that an amlodipine maleate and microcrystalline cellulose composition generally has a pH value of ca. 6. However such a one was suggested by US Patent Specification 4.590.195 (Annex ASt 3), Example 63 (solely

exchange of the effective agent there with R^4 for a residue, R^4 = hydrogen). In comparison with this established technology, in other words, the object of Patent Claim 2 of German Utility Model Specification 201 16 428, in particular with a pH value of ca. 6, lacks the inventive element.

9) **Patent Claim 3**

Also in US Patent Specification 5.155.120 (Annex ASt 1). The composition can exist in a solid form. E.g. in the form of tablets or capsules (see for example column 2 lines 43 to 57). This is also the case in US Patent Specification 6.057.344 (Annex ASt 5), in particular column 11 line 62 to column 12 line 24.

9) *(sic)* Patent Claims 4 to 7

a) Moreover in US Patent Specification 5.155.120 (Annex ASt 1), in particular column 2 line 46, the adjuvans that can be used is dicalcium phosphate = calcium hydrogen phosphate as per Patent Claims 4 and 6 of German Utility Model Specification 201 16 428.

In this regard, in other words, the objects of Patent Claims 4 and 6 of German Utility Model Specification 201 16 428 are not new.

They are also not based on an inventive element due to US Patent Specification 4.590.195 (Annex ASt 3), in particular Example 62.

b) In addition, in US Patent Specification 6.057.344 (Annex ASt 5), in particular column 11, line 44, microcrystalline cellulose as cited in Patent Claims of German Utility Model Specification 201 16 428 can be used as an adjuvans.

In this regard, in other words, the objects of Patent Claims 4 and 7 of German Utility Model Specification 201 16 428 are not new.

Moreover the use of microcrystalline cellulose, as per Patent Claims 4 and 7 of German Utility Model Specification 201 16 428, due to US Patent Specification 4.590.195 (Annex ASt 3), in particular Example 63, is not an inventive element.

c) In addition the use of calcium phosphate and microcrystalline cellulose, as per Patent Claim 5 of German Utility Model Specification 201 16 428, aside from the fact that this combination is standard technology, is not an inventive element due to US Patent Specification 4.879.303 (Annex ASt 4), in particular Example 2 in connection with Table 1 in column 2.

10) Patent Claim 8

In the light of page 8, line 4 below to the last line of German Utility Model Specification 201 16 428, acid pH adjusting agents are pharmaceutically acceptable acids. The algin acid cited in column 2 line 47 of US Patent Specification 5.155.120 (Annex ASt 1) exercises this function alongside its bursting effect.

Moreover the object of Patent Claim 8 of German Utility Model Specification 201 16 428, due to US Patent Specification 5.155.120 (Annex ASt 1) is not new.

In the light of page 9 lines 1 to 3 of German Utility Model Specification 201 16 428, pH regulators (pH adjusters) also include buffers. These are mentioned in Specification WO 95/34299 (Annex ASt 2), in particular on page 9, lines 25 to 26.

Moreover the object of Patent Claim 8 of German Utility Model Specification 201 16 428 is not therefore not new in the light of Specification WO 95/34299.

11) Patent Claim 9

Moreover, under US Patent Specification 5.155.120 (Annex ASt 1), in particular column 2 lines 43 to 52, the composition may take the form of a tablet.

Thus the object of Patent Claim 9 of German Utility Model Specification 201 16 428, in the light of US Patent Specification 5.155.120 (Annex ASt 1) is not new.

This is also the case in US Patent Specification 6.057.344 (Annex ASt 5), in particular column 11 line 62 to column 12 line 24. In addition the object of Patent Claim 9 of German Utility Model Specification 201 16 428 is, in other words, not new in the light of US Patent Specification 6.057.344 (Annex ASt 5).

12) Patent Claim 10

The provision of an outer moisture and/or light barrier layer is a routine measure by the specialist in the tablet field.

Moreover the outer moisture barrier layer is cited on page 11, para. 2 of German Utility Model Specification 201 16 428. This is in other words a feature devoid of any inventive element since US Patent Specification 6.057.344 (Annex ASt 5), in particular column 11, lines 52 to 55 cites the coating of amlodipine salt tablets.

13) Patent Claim 11

The composition in the form of a capsule was known from US Patent Specification 5.155.120 (Annex ASt 1), in particular column 2 lines 52 to 57 and US Patent Specification 6.057.344 (Annex ASt 5), in particular column 11, lines 47 and 63.

Thus the object of Patent Claim 11 of German Utility Model Specification 201 16 428 is not new.

14) Patent Claim 12

US Patent Specification 5.155.120 (Annex ASt 1), Patent Claim 2 cites a standard dosage of 10 mg amlodipine.

This falls within the range of 1.0 to 25 mg of free amlodipine base in Patent Claim 12 of German Utility Model Specification 201 16 428.

Thus Patent Claim 12 of German Utility Model Specification 201 16 428, due to US Patent Specification 5.155.120 (Annex ASt 1), is not new.

b) US Patent Specification 6.057.344 (Annex ASt 5), column 12 lines 18 to 19 cites a volume range between ca. 0.01 mg and ca. 50 mg amlodipine for the tablets and column 12 lines 20 to 21 cites a range of ca. 0.5 and ca. 50 mg amlodipine for the capsules. These ranges are covered the range of 1.0 to 25 mg cited in Patent Claim 12 of German Utility Model Specification 201 16 428.

Thus the object of Patent Claim 12 of German Utility Model Specification 201 16 428, in the light of US Patent Specification 6.057.344 (Annex ASt 5) are not new, or at least does not contain an inventive element.

c) US Patent Specification 4.590.195 (Annex ASt 3), column 5, lines 63 to 66 cites a content for the tablets or capsules of 1 to 10 mg of active agent differing slightly from amlodipine, this range falling within the range of 1.0 to 25 mg free amlodipine base cited in Patent Claim 12 of German Utility Model Specification 201 16 428, so that this Patent Claim 12, in the light of US Patent Specification 4.590.195 (Annex ASt 3) does not contain an inventive element.

15) Patent Claim 13

a) As can be seen from the above Point 14 a), the volume alternative of 10 mg free amlodipine base in Patent Claim 13 of German Utility Model Specification 201 16 428 is not new in the light of US Patent Specification 5.155.120 (Annex ASt 1).

b) As can be seen from the above Points 14 b) and c), US Patent Specification 6.056.244 (Annex ASt 5) and US Patent Specification 4.590.195 (Annex ASt 3) also include standard dosages of 1.25, 2.5 or 5 mg of free amlodipine base.

In this regard your attention is also drawn to column 12, lines 21 to 24 of US Patent Specification 6.057.344 (Annex ASt 5) with details of standard dosages of 2.5 and 5.0 mg.

Moreover, in relation to standard dosages of 1.25, 2.5 and 5 mg, Patent Claim 13 of German Utility Model Specification 201 16 428 is not new nor does in contain an inventive element.

16) **Patent Claim 14**

The content of this Patent Claim is objectively the same as Patent Claim 1, which has already been shown not to be new, since the mixture of the adjuvans(s) with the amlodipine maleate, which, as a procedural feature is not subject to utility model protection, of necessity leads to the mixture of the same under Patent Claim 1 of German Utility Model Specification 201 16 428.

17) **Patent Claim 15**

The object of this Patent Claim 15 is just as not new as that of Patent Claim 9, the non-newness of which being demonstrated in Point 11 above. The pressing as the inadmissible utility model protection feature does not alter the features of the tablet. Moreover this pressing, even if it were relevant, I he

light of column 12, lines 12 to 16 of US Patent Specification 6.057.344 (Annex ASt 5) does not represent an inventive measure.

18) Patent Claim 16

The object of this Patent Claim 16 is just as not new as that of Patent Claim 11, the non-newness of which was demonstrated in Point 13 above. The pouring of the mixture into a capsule as the utility model feature to be protected does not change the features of the capsule. Moreover this pouring into capsules, even if it were relevant, is not new in the light of US Patent Specification 5.155.120 (Annex ASt 1), column 2, lines 53 to 57.

19) Patent Claims 17 and 18

The mixing using moist granulation or a dry process also, like mixing per se, does not change the tablet's material features as defined in Patent Claim 9, the non-newness of which was demonstrated in Point 11.

20) Patent Claims 19 and 20

These Patent Claims, only consisting of procedural features, are formally inadmissible for a utility model. Moreover these concern normal particle sizes devoid of any inventive element.

The cancellation petition fee of €300.00 has been paid.

Signed Dr. Beszédes
Patent Lawyer

Annexes

Copies of this cancellation petition dated January 27th 2003	duplicate
Copies of the German Utility Model Specification DE 201 16 428 U1 (Annex ASt A)	triplicate
Copies of US Patent Specification 5.155.120 (Annex ASt 1)	triplicate
Copies of Specification WO 95/34299 (Annex ASt 2)	triplicate
Copies of US Patent Specification 4.590.195 (Annex ASt 3)	triplicate
Copies of US Patent Specification 4.879.303 (Annex ASt 4)	triplicate

Copies of US Patent Specification 6.057.344
(Annex ASt 5) triplicate

List of Annexes triplicate

Le

Ast A

(19) BUNDESREPUBLIK DEUTSCHLAND

DEUTSCHES PATENT- UND MARKENAMT

(12) **Gebrau hsmusterschrift**

(10) **DE 201 16 428 U 1**

(51) Int. Cl.[7]: A 61 K 31/44

(21) Aktenzeichen: 201 16 428.0
(22) Anmeldetag: 8. 10. 2001
(47) Eintragungstag: 10. 1. 2002
(43) Bekanntmachung im Patentblatt: 14. 2. 2002

DE 201 16 428 U 1

(30) Unionspriorität:
60/258,562 29. 12. 2000 US
09/809,346 16. 03. 2001 US

(73) Inhaber:
BioOrganics B.V., Nijmegen, NL

(74) Vertreter:
W. Maiwald und Kollegen, 20099 Hamburg

(54) Pharmazeutische Zusammensetzungen umfassend Amlodipinmaleat

(57) Pharmazeutische Zusammensetzung, umfassend eine wirksame Menge an Amlodipinmaleat und mindestens einen pharmazeutisch annehmbaren Hilfsstoff, wobei die Zusammensetzung einen pH-Wert innerhalb eines Bereich von 5,5–7,0 hat.

DE 201 16 428 U 1

09.10.01

MAIWALD PATENTANWALTS GMBH

Hamburg · München · Düsseldorf

Patentanwälte

Dr. Walter Maiwald (München)
Dr. Volker Hamm (Hamburg)
Dr. Stefan Michalski (Düsseldorf)
Dr. Regina Neuefeind (München)
Dipl.-Ing. Univ. Udo Preuss (München)

Rechtsanwalt
Stephan N. Schneller (München)

In Kooperation mit:
Dr. Schmidt-Felzmann & Kozianka
Rechtsanwälte
(Hamburg)

Parr · Tauche ·
Leutheusser-Schnarrenberger
Rechtsanwälte
(München · Starnberg)

Aktenzeichen	Unser Zeichen	Hamburg,
Gebrauchsmuster-anmeldung	B 2006 / VH	08. Oktober 2001

BioOrganics B.V.

Microweg 22, 6545 CM Nijmegen, Niederlande

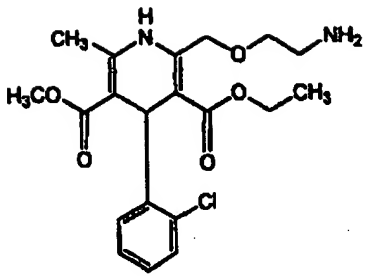
Pharmazeutische Zusammensetzungen umfassend Amlodipinmaleat

Die vorliegende Erfindung betrifft pharmazeutische Zusammensetzungen, die Amlodipinmaleat umfassen.

Calciumkanalblocker können für die Behandlung einer Vielzahl von kardialen Erkrankungen, insbesondere Angina und Hochdruck, verwendet werden. EP 089 167 und das entsprechende US 4 572 909 offenbaren eine Klasse von substituierten Dihydropyridin-Derivaten als brauchbare Calciumkanalblocker. In diesen Patenten wird als eine der am meisten bevorzugten Verbindungen 2-[(2-Aminoethoxy)methyl]-4-(2-chlorphenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin angegeben. Diese Verbindung, die nun allgemein als Amlodipin bekannt ist, hat die nachfolgende Strukturformel:

VH:SHB:hf

Postfach 113417 · 20434 Hamburg · Ballindamm 37 · 20095 Hamburg
Tel. +49 (0)40 555 895 0 · Fax +49 (0)40 555 405 25 · http://www.maiwald.de · hamm@maiwald.de
Geschäftsführer: Dr. Walter Maiwald · Dr. Volker Hamm · Dr. Stefan Michalski · HRB Nr. 71405



Die Beispiele 8, 11, 12 und 22 des EP 089 167 zeigen die Synthese des Amlodipins in der Maleatsalzform. Während in dem genannten Patent eine Vielzahl von Säureadditionssalzen gelehrt wird, wird das Maleatsalz als das am meisten bevorzugte Säureadditionssalz angegeben.

Nachfolgend wurden das EP 244 944 und das entsprechende US 4 879 303 betreffend das Besylat (oder Benzolsulfonat)-Salz des Amlodipins veröffentlicht. In diesen Patenten wird angegeben, daß das Besylatsalz gegenüber den vorbekannten Salzen bestimmte Vorteile zeigt, wie etwa gute Eigenschaften bei der Formulierung. Tatsächlich wurde das Amlodipinbesylat und nicht das Amlodipinmaleat für ein kommerzielles, verschreibungspflichtiges Arzneimittel von Pfizer verwendet, welches in den USA unter der Handelsbezeichnung NORVASC vertrieben wird.

Eine Überprüfung der zugänglichen Teile betreffend die NORVASC (Amlodipinbesylat) New Drug Application (NDA), angemeldet bei der U.S. Food & Drug Administration durch Pfizer, ergibt, daß während der Entwicklung vom ursprünglichen Amlodipinmaleat auf das Amlodipinbesylat umgeschwenkt wurde (siehe „Review of Original NDA" für NDA# 19-787 vom 10. Oktober 1990, das von der FDA gemäß dem Freedom of Information Act erhalten werden kann). Die Gründe für das Umschwenken waren offensichtlich Tablettier- und Stabilitätsprobleme. Jedoch sind die genauen Stabilitäts- und Tablettierprobleme/aspekte/ursachen nicht in den von der FDA erhältlichen Informationen öffentlich offenbart. Interessanterweise umfassen die klinischen Studien gemäß der NDA die Verwendung der Maleatsalzform und der Besylatsalzform, wobei die beiden Salzformen therapeutisch äquivalent (bio-

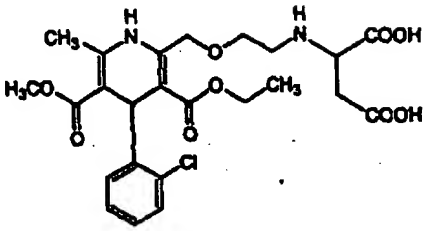
äquivalent) sind. Jedoch wurde in diesen Studien Amlodipinmaleat immer in einer Darreichungsform in Form von Kapseln oder Lösungen, nicht in Form von Tabletten verwendet.

Die vorliegende Erfindung betrifft das Auffinden stabiler, Amlodipinmaleat enthaltender pharmazeutischer Zusammensetzungen. Ein erster Aspekt der Erfindung betrifft eine pharmazeutische Zusammensetzung, die eine wirksame Menge an Amlodipinmaleat sowie mindestens einen pharmazeutisch annehmbaren Hilfsstoff umfaßt, wobei die Zusammensetzung einen pH-Wert innerhalb eines Bereiches von 5,5 - 7,0 aufweist. Die Zusammensetzung ist vorzugsweise eine feste Darreichungsform wie etwa eine Tablette oder eine Kapsel.

Ein weiterer Aspekt der Erfindung betrifft eine pharmazeutische Zusammensetzung, die durch ein Verfahren herstellbar ist, bei dem feste Partikel von Amlodipinmaleat, die eine durchschnittliche Partikelgröße von mindestens 20 µm haben, mit mindestens einem pharmazeutisch annehmbaren Hilfsstoff vermischt werden. Die Mischung hat vorzugsweise einen pH-Wert zwischen 5,5 und 7,0. Die Mischung kann zu Tabletten verpreßt oder in Kapseln abgefüllt werden, um eine feste Darreichungsform herzustellen.

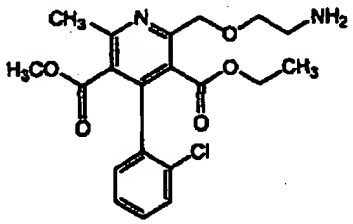
Die Stabilität ist ein wichtiger Aspekt für eine pharmazeutische Zusammensetzung. Die vorliegende Erfindung basiert auf der Erkenntnis, daß die vorbekannten, mit Amlodipinmaleat verbundenen Stabilitätsprobleme hauptsächlich dadurch überwunden werden können, daß der pH-Wert der Zusammensetzung dahingehend kontrolliert wird, daß er in einem Bereich zwischen etwa 5,5 und 7,0, vorzugsweise zwischen etwa 6,0 und 7,0 liegt. Innerhalb dieses Bereiches werden mögliche Abbaureaktionen vermindert. Im besonderen wird die Bildung des folgenden Abbauproduktes, hier als Amlodipinasparat bezeichnet, in diesem pH-Bereich vermindert oder verhindert.

Amlodipinaspartat hat folgende Strukturformel:



Amlodipinaspartat wird durch eine Michael-Additionsreaktion zwischen Amlodipin und Maleinsäure gebildet. Da Amlodipin und Maleinsäure im Amlodipinmaleat-Salz miteinander in engem Kontakt sind, nimmt die Wahrscheinlichkeit, daß die Additionsreaktion stattfindet, mit der Zeit zu; folglich ergibt sich ein Stabilitätsaspekt. Durch die Kontrolle des pH-Wertes wird die Addition deutlich verlangsamt oder gänzlich verhindert. Somit ist nun gefunden worden, daß ein über 7,0 liegender pH-Wert tendenziell den Abbau des Amlodipinmaleats in das Amlodipinaspartat begünstigt. Unter einem pH-Wert von etwa 5,5 werden andere Abbaureaktionen begünstigt, die das Pyridin-Analogon von Amlodipin umfassen, welches folgende Strukturformel hat:

„Amlo-Pyridin"



Vorzugsweise liegt der pH-Wert der stabilisierten Zusammensetzung der vorliegenden Erfindung in einem Bereich zwischen etwa 6,0 und 7,0 und typischerweise zwischen etwa 6,1 oder 6,2 und 6,8. Für feste Zusammensetzungen wird der pH-Wert bestimmt, indem eine Aufschlämmung des Materials mit Wasser (entmineralisiertes Wasser) gebildet wird und der pH-Wert der Aufschlämmung gemessen wird, wie es sich für den Fachmann hinsichtlich des pH-

Werts einer festen Zusammensetzung versteht. Die Konzentration der Zusammensetzung in der Aufschlämmung beträgt 20 Gew.-%. Der pH-Wert wird durch eine beliebige Standard-Technik gemessen.

Die pharmazeutische Zusammensetzung der vorliegenden Erfindung umfaßt eine pharmazeutisch wirksame Menge an Amlodipinmaleat und mindestens einen pharmazeutisch annehmbaren Hilfsstoff. Vorzugsweise ist die Stabilität der Zusammensetzung derart, daß sie nach drei Monaten, vorzugsweise nach sechs Monaten, in einem umgebungskontrollierten Raum bei 40°C/75% RF einen Verlust an Amlodipin (oder dementsprechend eine Zunahme des Gehaltes an Verunreinigungen) von weniger als 10%, vorzugsweise von weniger als 5% und bevorzugter von weniger als 1% zeigt. Alternativ zeigt das Amlodipinmaleat der pharmazeutischen Zusammensetzung der vorliegenden Erfindung insbesondere eine Lagerstabilität, die derjenigen von Amlodipinbesylat-Zusammensetzungen entspricht oder besser als diese ist. Zum Beispiel ist der Verlust an Amlodipin aufgrund von Zersetzungsreaktionen während der Lagerung gleich (+/- 10%) oder geringer als der Amlodipinverlust bei Amlodipinbesylat-Zusammensetzungen, insbesondere dem kommerziellen Produkt.

Die Form des Amlodipinmaleats ist nicht irgendwie begrenzt und umfaßt Anhydrate, Solvate, Hydrate und partielle Hydrate, als auch kristalline und amorphe Formen. Des weiteren kann das Verhältnis des Amlodipins zum Maleat verändert werden und umfaßt insbesondere die üblichere und vorbekannte Form von 1:1, als auch eine neue 2:1-Form, die in der US-Patentanmeldung Nr. 09/809,356, angemeldet am 16. März 2001, der vorliegenden Anmelderin mit dem Titel „Amlodipin Hemimaleat" beschrieben wird.

Die Menge an Amlodipin ist nicht irgendwie begrenzt und umfaßt jede beliebige Menge, die eine pharmazeutische Wirkung hervorruft. Insbesondere kann Amlodipinmaleat zur Behandlung oder Vorbeugung von Hochdruck oder Angina verwendet werden, indem eine wirksame Menge davon an einen Patienten verabreicht wird, der dessen bedarf. Die spezifische Anginaform ist nicht irgendwie begrenzt und schließt insbesondere die chronische stabile Angina pectoris und die vasospastische Angina (Prinzmetal-Angina) ein. Die Verbindung kann über

jeden geeigneten Weg verabreicht werden, beispielsweise oral oder parenteral. Die zu behandelnden „Patienten" sind Menschen und nicht-menschliche Tiere, insbesondere nicht-menschliche Säugetiere. Die wirksame Menge an Amlodipinmaleat in einer Einzeldosiseinheit liegt im allgemeinen im Bereich zwischen 1 und 100 mg, üblicherweise zwischen 1 und 25 mg und insbesondere bei 1, 1,25, 2,5, 5 oder 10 mg (bezogen auf die freie Base).

Amlodipinmaleat kann durch beliebige bekannte Techniken gemäß dem Stand der Technik hergestellt werden, einschließlich derjenigen, die in den oben genannten Patenten EP 089 167 und US 4 572 909 beschrieben sind. Es ist erwünscht, daß das wirksame Amlodipinmaleat weitgehend rein ist. Beispielsweise sollte der Anteil an Verunreinigungen, wie etwa Amlodipinaspartat, Amlopyridin, etc., die während der Synthese gebildet werden, begrenzt werden, vorzugsweise auf weniger als 2 Gew.-%, jedoch ist eine solche Reinheit für die vorliegende Erfindung nicht erforderlich. Ein geeignetes Verfahren, Amlodipinmaleat im wesentlichen frei von Amlodipinaspartat herzustellen, wird in der US-Patentanmeldung Nr. 09/809,343, angemeldet am 16. März 2001, der vorliegenden Anmelderin mit dem Titel „Process for Making Amlodipine Maleate" beschrieben. In ähnlicher Weise wird ein geeignetes Verfahren zur Herstellung einer freien Amlodipinbase in der US-Patentanmeldung Nr. 09/809,351, angemeldet am 16. März 2001, der vorliegenden Anmelderin mit dem Titel „Process for Making Amlodipine, Derivatives Thereof, and Precursors Therefor" beschrieben.

Die pharmazeutischen Zusammensetzungen der vorliegenden Erfindung umfassen auch mindestens einen Hilfsstoff. Ein „Hilfsstoff", wie er hierin verwendet wird, bedeutet eine beliebige pharmazeutisch annehmbare unwirksame Komponente der Zusammensetzung. Wie bekannt, schließen Hilfsstoffe Streckmittel, Bindemittel, Gleitmittel, Sprengmittel, Farbstoffe, Konservierungsstoffe, pH-Einsteller etc. ein. Die Hilfsstoffe werden auf der Basis der gewünschten physikalischen Aspekte der Endform ausgewählt, beispielsweise um eine Tablette mit gewünschter Härte und Friabilität, eine schnell dispergierbare oder leicht schluckbare Tablette etc. zu erhalten. Die erwünschte Freisetzungsgeschwindigkeit der wirksamen Substanz aus der Zusammensetzung nach ihrer Einnahme spielt bei der Auswahl der Hilfsstoffe eben-

falls eine Rolle. Die bevorzugte Freisetzungsgeschwindigkeit ist die mit kommerziell erhältlichen Amlodipinbesylat-Tabletten vergleichbare Geschwindigkeit.

Geeignete Hilfsstoffe für die Verwendung gemäß der Erfindung umfassen:

- ein Streckmittel wie etwa Calciumhydrogenphosphat, Lactose, Mannitol etc.
- ein Bindemittel wie etwa mikrokristalline Cellulose oder modifizierte Cellulose, Povidon etc.
- ein Sprengmittel wie etwa Natriumstärkeglykolat, Crosspovidon
- ein Gleitmittel wie etwa Magnesiumstearat, Natriumstearylfumarat, Talk
- ein Farbstoff, Geschmacksmaskierungsmittel etc.

Der pH-Wert der Zusammensetzung kann durch die geeignete Auswahl an Hilfsstoffen kontrolliert oder eingestellt werden. Es sollte berücksichtigt werden, daß Amlodipinmaleat leicht sauer ist. Beispielsweise hat Amlodipinmaleat als eine gesättigte wäßrige Lösung einen pH-Wert von etwa 4,8. Folglich führt die Verwendung von Hilfsstoffen, die pH-inert sind, d.h. einen geringen bis keinen Effekt auf den pH-Wert ausüben, im allgemeinen zu einer nicht-alkalischen pharmazeutischen Zusammensetzung, da das Amlodipinmaleat hauptsächlich als sein eigenes Einstellmittel agiert. Ein Beispiel für einen pH-inerten Hilfsstoff ist mikrokristalline Cellulose. Eine Amlodipinmaleat und mikrokristalline Cellulose umfassende Zusammensetzung zeigt im allgemeinen einen pH-Wert von etwa 6. Zum Vergleich besitzt die entsprechende Amlodipinbesylat-Zusammensetzung im allgemeinen einen pH-Wert von etwa 7 und die entsprechende freie Amlodipinbase-haltige Zusammensetzung im allgemeinen einen pH-Wert von etwa 9. Kommerziell erhältliche, Amlodipinbesylat umfassende und unter der Markenbezeichnung Norvasc vertriebene Tabletten zeigen einen pH-Wert von typischerweise zwischen 7,05-7,35 (wiederum gemessen als eine 20 Gew.-%-ige Aufschlämmung).

Es können auch Hilfsstoffe verwendet werden, die einen Effekt auf den pH-Wert ausüben. Der pH-Wert dieser Hilfsstoffe muß bei der Herstellung der pharmazeutischen Zusammensetzung berücksichtigt werden, so daß der Gesamt-pH-Wert der pharmazeutischen Zusammensetzung in einem Bereich zwischen etwa 5,5 und 7,0 liegt.

Beispielsweise sind kommerziell erhältliche/pharmazeutisch annehmbare Calciumphosphate im allgemeinen alkalisch, d.h. sie haben einen pH-Wert größer als 7, wenn er wie zuvor beschrieben in einer 20%-igen Aufschlämmung gemessen wird. Zum Beispiel wird angegeben, daß DI-TAB, ein kommerziell erhältliches doppelbasisches Calciumphosphatdihydrat einen pH-Wert von etwa 7,4 besitzt. Dennoch sind einige Formen und Arten von Calciumphosphat pH-sauer oder -neutral. Dieser niedrige pH-Wert kann aufgrund der Spezies des Calciumphosphates und auch durch die Behandlung während der Herstellung des Materials, wie etwa durch die Entfernung von Verunreinigungen/ Waschen, herrühren. Zum Beispiel gilt allgemein, daß doppelbasisches Calciumphosphatanhydrat einen pH-Wert von etwa 7,3 hat, wobei A-Tab ™ (Rhodia), ebenfalls ein doppelbasisches Calciumphosphatanhydrat, einen pH-Wert von etwa 5,0 hat. Weitere Beispiele kommerziell erhältlicher, nicht-alkalischer Calciumphosphate umfassen DiCAFOS A (Budenheim) mit einem pH-Wert von etwa 7 (10%-ige Aufschlämmung) und Fujicalin SG (Fuji) mit einem pH-Wert zwischen 6,1-7,2 (5%-ige Aufschlämmung). Bei Verwendung eines nicht-alkalischen Calciumphosphates als Hilfsstoff kann eine pharmazeutische Zusammensetzung erlangt werden, die den erwünschten pH-Wert aufweist. Alternativ kann eine Mischung von Calciumphosphaten, einige mit einem pH-Wert über 7 und einige mit einem pH-Wert unter 7, verwendet werden, um den erwünschten pH-Wert der Zusammensetzung zu erreichen.

Anstelle von oder zusätzlich zu den nicht-alkalischen Calciumphosphaten können andere saure Träger- oder Hilfsstoffe an sich oder um den alkalischen Hilfsstoff auszugleichen verwendet werden. Ein Beispiel für einen solchen sauren Hilfsstoff ist das Sprengmittel Explotab(TM) von Penwest, welches ein vernetztes, gering substituiertes Natriumstärkeglykolat ist. Des weiteren können auch den pH-Wert einstellende Mittel benutzt werden, um den erwünschten pH-Wert zu erreichen. Diese Mittel umfassen pharmazeutisch annehmbare Säuren, wie etwa Maleinsäure, Zitronensäure oder Ascorbinsäure (die letzten beiden können auch als Antioxidantien agieren) und pharmazeutisch annehmbare Basen, wie etwa Calciumoxid

oder Magnesiumoxid. Salze von schwachen Säuren und/oder von schwachen Basen sind auch geeignete pH-Regulierer, da sie als Puffer agieren, indem sie den pH-Wert gemäß der chemischen Natur ihrer Komponenten erniedrigen oder erhöhen.

Die pharmazeutischen Zusammensetzungen der vorliegenden Erfindung sind hinsichtlich der Form oder des Wegs der Verabreichung nicht irgendwie begrenzt. Orale Darreichungsformen und auch parenterale Darreichungsformen sind einbezogen. Die Zusammensetzung kann in Form einer Flüssigkeit, eines Feststoffes oder einer Suspension vorliegen. Vorzugsweise ist die pharmazeutische Zusammensetzung eine feste Darreichungsform, wie etwa eine Tablette, eine Kapsel oder ein Beutel, die zur oralen Verabreichung vorgesehen sind.

Bevorzugte feste Darreichungsformen enthalten als hauptsächlichen Hilfsstoff mikrokristalline Cellulose, ein Calciumphosphat, insbesondere Calciumhydrogenphosphat, oder Kombinationen dieser. Die Summe an anderen Hilfsstoffen, falls vorhanden, beträgt im allgemeinen weniger als 25 Gew.-%, üblicherweise weniger als 10 Gew.-% und in einigen Fällen weniger als 5 Gew.-% der gesamten pharmazeutischen Zusammensetzung. Andere bevorzugte Hilfsstoffe sind Sprengmittel, wie etwa Natriumstärkeglykolat und/oder Schmiermittel, wie etwa Magnesiumstearat und/oder Talk.

Beispielsweise zeigt sich für eine Amlodipinmaleat und mikrokristalline Cellulose als einzigen Hilfsstoff umfassende pharmazeutische Zusammensetzung eine gegenüber der Bildung von Verunreinigungen gute Stabilität.

Die pharmazeutischen Zusammensetzungen der vorliegenden Erfindung können mittels im Fachgebiet allgemein bekannter Techniken hergestellt werden. Im allgemeinen wird Amlodipinmaleat mit einem oder mehreren Hilfsstoffen vermischt, um eine Mischung zu bilden. Das Vermischen kann feucht oder trocken durchgeführt werden (d.i., indem ein Lösungsmittel oder ein flüssiges Streckmittel während der Verarbeitung verwendet oder nicht verwendet wird) und kann das Granulieren, Verwirbeln oder Vermischen von Pulvern umfassen. Ein trockenes Verfahren ist jedoch bevorzugt. Die Mischung kann, nach wahlweiser Weiterver-

arbeitung, zu Tabletten verpreßt oder in Kapseln wie etwa Gelatinekapseln abgefüllt werden. Typischerweise ist das zu vermischende Amlodipinmaleat in der Form von Partikeln. Die Lagerstabilität der pharmazeutischen Zusammensetzung der vorliegenden Erfindung wird im allgemeinen gesteigert, indem größere Partikelgrößen verwendet werden. Vorzugsweise ist die durchschnittliche Partikelgröße des Amlodipinmaleats mindestens 20 µm, üblicherweise mindestens 100 µm und in einigen Ausführungsformen mindestens 300 µm. Falls eine Einstellung des pH-Werts der Zusammensetzung erforderlich ist, wird er eingestellt, bevor die Zusammensetzung zur Endform, wie etwa zur Tablette oder zur Kapsel, verarbeitet wird.

Beispielsweise können erfindungsgemäße Tabletten mittels einer Feuchtgranulation einer Mischung aus Amlodipinmaleat und einem festen Hilfsstoff/Streckmittel wie etwa Calciumphosphat des geeigneten Grades, mit Hilfe eines Granulier-Lösungsmittels, wie etwa Wasser oder Ethanol, Trocknen des feuchten Granulates, Sieben des Granulates, Vermischen mit Natriumstärkeglykolat und Magnesiumstearat und Verpressen der Tablettenmischung zu Tabletten hergestellt werden. Die Kontrolle des pH-Werts und/oder das Einstellen des pH-Werts sollte günstigerweise durchgeführt werden, bevor mit Magnesiumstearat vermischt wird. In diesem Beispiel werden sowohl der Granulierschritt als auch der Mischschritt als „Vermischen" betrachtet, da Amlodipinmaleat und ein Hilfsstoff vermischt werden.

Ein weiteres geeignetes Verfahren umfaßt die direkte Verpressung der Mischung aus Amlodipinmaleat und dem/mindestens einen Hilfsstoff. Bei diesem Verfahren werden die Inhaltsstoffe zusammengemischt, um eine preßbare Mischungszusammensetzung zu bilden, die anschließend zu einer Tablette verpreßt wird. Eine Mischung, die Amlodipinmaleat, mikrokristalline Cellulose und/oder Calciumphosphat und gegebenenfalls Natriumstärkeglykolat und/oder Magnesiumstearat umfaßt, kann zur Herstellung einer Tablette mittels direkter Verpressung geeignet sein. Beispielsweise kann eine Amlodipinmaleat, Calciumhydrogenphosphat, mikrokristalline Cellulose und Natriumstärkeglykolat enthaltende Mischung, die einen pH-Wert zwischen 5,5 und 7,0 hat, zusammengemischt, erneut mit Magnesiumstearat gemischt und zu einer stabilen Tablette verpreßt werden.

Die Amlodipinmaleat, mikrokristalline Cellulose, Natriumstärkeglykolat und Magnesiumstearat, und gegebenenfalls zusätzlich Calciumhydrogenphosphat umfassenden Tabletten der vorliegenden Erfindung zeigen nicht das Problem, daß sie an dem Tablettenstempel kleben, wie es im Stand der Technik für andere Amlodipinformulierungen berichtet wird (siehe zuvor genanntes Patent EP 244 944). Demgemäß kann die Zusammensetzung der vorliegenden Erfindung in industriellem Maßstab ohne technische Probleme hergestellt werden.

Die Tabletten können mit einer geeigneten Beschichtung überzogen werden. Beispielsweise kann die Beschichtung eine Feuchtigkeitssperre sein, um die Lagerstabilität zu unterstützen oder eine Beschichtung für die hinhaltende oder verzögerte Freisetzung sein, wie sie im Fachgebiet bekannt sind.

Alternative Darreichungsformen sind Kapseln, sowohl weiche als auch harte Kapseln. Die stabilisierte Amlodipinmaleat-Zusammensetzung der Erfindung, wie sie zuvor beschrieben worden ist, wird durch bekannte Techniken in Kapseln abgefüllt, in Mengen, die die gewünschte therapeutische Dosis des Amlodipins umfassen.

Geeignete Materialien zur Verpackung der pharmazeutischen Darreichungsformen sind Kunststoff- oder Glasbehälter und Blister. Insbesondere aus nicht-permeablen Materialien (Polyethylen hoher Dichte oder Aluminium) hergestellte Blister sind vorteilhaft, da sie dazu beitragen können, die Bildungsgeschwindigkeit von Abbau-Verunreinigungen, nämlich die Amlopyridin-Verunreinigung, während der Lagerung zu verringern.

Die pharmazeutischen Zusammensetzungen der vorliegenden Erfindung werden, wie zuvor schon erwähnt, zur Behandlung oder Vorbeugung von Angina oder Hochdruck verwendet, indem eine wirksame Menge der pharmazeutischen Zusammensetzung an einen Patienten verabreicht wird, der dessen bedarf. Die pharmazeutische Zusammensetzung ist typischerweise eine Einzeldosiseinheit. Einzelne Einzeldosis-Zusammensetzungen enthalten im allgemeinen zwischen 1 bis 100 mg, üblicher zwischen 1 bis 25 mg Amlodipinmaleat. Bevorzugt sind Einzeldosiseinheiten, umfassend Amlodipinmaleat in einem Äquivalent von 1,25, 2,5, 5

oder 10 mg an Amlodipin, wie etwa Tabletten oder Kapseln zur oralen Verabreichung. Die pharmazeutische Zusammensetzung wird täglich 1 bis 3 mal, vorzugsweise 1 mal täglich verabreicht. Die obigen Zusammensetzungen können auch für die Verminderung der Symptome eines Herzversagens, zur Verbesserung der systolischen links-ventrikulären Funktion und zur Steigerung der Leistungsfähigkeit bei Patienten mit ischaemischer LVD und Herzversagen ohne Angina verwendet werden.

Erfindungsgemäße Amlodipinmaleat-Zusammensetzungen können auch für medizinische Anwendungen in Kombination mit anderen Antihypertensiva und/oder Antianginosa verwendet werden, z.B. mit ACE-Hemmern, wie beispielsweise Benazepril. Die Kombination kann in Form eines einzelnen Kombinationspräparates, z.B. als eine Amlodipinmaleat und Benazeprilhydrochlorid enthaltende Kapsel, oder durch separate Verabreichung von Arzneimitteln enthaltend die obigen Wirkstoffe realisiert werden.

In ähnlicher Weise kann Amlodipinmaleat auch mit HMG-CoA-Reduktasehemmern, insbesondere Statinen, wie Lovastin, Simvastatin, Atorvastatin oder dergleichen, kombiniert werden.

Beispiele

Beispiel 1 Amlodipinmaleat-Tabletten auf der Basis von Calciumphosphat-Hilfsstoffen

a) Tablettenzusammensetzungen umfassend Calciumphosphate verschiedener pH-Werte

Chargen-Nummer	(A)	(B)	(C)	(D)	(E)	(F)
Äquivalent an Amlodipin	2,5 mg	10 mg	2,5 mg	10 mg	2,5 mg	10 mg
Amlodipinmaleat	3,21 mg	12,8 mg	3,21 mg	12,8 mg	3,21 mg	12,8 mg
Wasserfreies Calciumhydrogenphosphat -DiCAFOS A - A-TAB - Fujicalin	31,5 mg	126,0 mg	31,5 mg	126,0 mg	31,5 mg	126,0 mg
Mikrokristalline Cellulose	62,05 mg	248,1 mg	62,05 mg	248,1 mg	62,05 mg	248,1 mg
Natriumstärkeglykolat	2,0 mg	8,0 mg	2,0 mg	8,0 mg	2,0 mg	8,0 mg
Magnesiumstearat	1,0 mg	4,0 mg	1,0 mg	4,0 mg	1,0 mg	4,0 mg
Gesamt	99,76 mg	398,9 mg	99,76 mg	398,9 mg	99,76 mg	398,9 mg

Typen von verwendeten wasserfreien Calciumhydrogenphosphaten

Chargen-Nummer	End-pH-Wert der 20% (m/V) Aufschlämmung der Tabletten	Typ des $CaHPO_4$	Lieferant des $CaHPO_4$	pH-Wert der 5% (m/V)-Aufschlämmung des $CaHPO_4$	pH-Wert der 20 % (m/V)-Aufschlämmung des $CaHPO_4$
(A), (B)	6,13 , 6,19	Di CAFOS A	Budenheim	7,29	6,69
(E), (F)	5,74, 5,74	Fujicalin	Fuji	6,12	5,62
(C), (D)	5,53, 5,54	A-TAB	Rhone-Poulenc	6,03	5,25

b) Tablettenzusammensetzungen umfassend Amlodipinmaleat verschiedener Partikelgrößen

Chargen-Nummer	(G)	(H)	(I)
Äquivalent an Amlodipinbase	2,5 mg	10 mg	2,5 mg
Amlodipinmaleat, zerkleinert	3,21 mg	12,8 mg	-
Amlodipinmaleat	-	-	3,21 mg
Wasserfreies Calciumhydrogenphosphat (pH 6,7)	31,5 mg	126,0 mg	31,5 mg
Mikrokristalline Cellulose	62,05 mg	248,1 mg	62,05 mg
Natriumstärkeglykolat	2,0 mg	8,0 mg	2,0 mg
Magnesiumstearat	1,0 mg	4,0 mg	1,0 mg
Gesamt	99,76 mg	398,9 mg	99,76 mg

Die Partikelgröße der Amlodipinmaleat-Substanz, die zur Herstellung der Chargen A-F verwerdet wurde, wurde mittels Laserdiffraktion gemessen und es wurde gezeigt, daß weniger als 90% der Partikel kleiner als 204 µm und 50% der Partikel kleiner als 80 µm sind.

Dieses Amlodipinmaleat wurde auf eine Partikelgröße von 10-20 µm zerkleinert und wurde zur Herstellung der Chargen (G) und (H) verwendet. Abwechselnd wurde eine weitere Charge von Amlodipinmaleat mit Partikelgrößen von 90% kleiner als 11 µm und 50% kleiner als 6 µm zur Herstellung der Charge (I) verwendet.

c) Herstellungsverfahren

Chargen (A) – (F) und (I) wurden wie folgt hergestellt:

- Das Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.

- Alle Hilfsstoffe außer Magnesiumstearat wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- 2,5 mg und/oder 10 mg-Tabletten wurden verpreßt, indem eine Korsch EK0-Exzenterpresse verwendet wurde.

Chargen (G) und (H) wurden wie folgt hergestellt:

- Amlodipinmaleat wurde zerkleinert.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Alle Hilfsstoffe außer Magnesiumstearat wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min gemischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- 2,5 mg/10 mg-Tabletten wurde auf einer Korsch EK0-Exzenterpresse verpreßt.

Beispiel 2 Tabletten umfassend mikrokristalline Cellulose

a) Zusammensetzung

Chargen-Nummer	(J)	(K)
Äquivalent an Amlodipinbase	2,5 mg	10 mg
Amlodipinmaleat	3,21 mg	12,8 mg
Mikrokristalline Cellulose	75,55 mg	302,1 mg
Vorgetrocknete Kartoffel-Stärke	20,0 mg	80,0 mg
Magnesiumstearat	0,5 mg	2,0 mg
Talk	0,5 mg	2,0 mg
Gesamt	99,76 mg	398,9 mg

Herstellungsverfahren

- Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Alle Hilfsstoffe außer Magnesiumstearat und Talk wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat und Talk wurden hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- 2,5 mg und 10 mg-Tabletten wurden verpreßt, indem eine Korsch EK0-Exzenterpresse verwendet wurde.

Eigenschaften der Tablettenzusammensetzung:

pH-Wert der 20% (w/V)-Aufschlämmung: - Charge (J) = 5,92

- Charge (K) = 5,96

Beispiel 3 Amlodipinmaleat-Tabletten umfassend Mannitol

Zusammensetzung

Chargen-Nummer	(L)
Äquivalent an Amlodipinbase	10 mg
Amlodipinmaleat	12,8 mg
Mannitol	370,1 mg
Natriumstärkeglykolat	8,0 mg
Magnesiumstearat	6,0 mg
Talk	2,0 mg
Gesamt	398,9 mg

Herstellungsverfahren

- Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.

- Alle Hilfsstoffe außer Magnesiumstearat und Talk wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat und Talk wurden hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- 10 mg-Tabletten wurden verpreßt, indem eine Korsch EK0-Exzenterpresse verwendet wurde.

Beispiel 4 Amlodipinmaleat-Tabletten, die einen pH-Wert größer als 7 haben (Vergleichsbeispiel)

Zusammensetzung (pH-Wert der 20% w/v-Aufschlämmung: 8,68)

Chargen-Nummer	(X)
Äquivalent an Amlodipinbase	2,5 mg
Amlodipinmaleat Wasserfreies Calciumhydrogenphosphat (pH 6,69)	3,21 mg 31,5 mg
Magnesiumoxid Ponderosum	0,5 mg
Mikrokristalline Cellulose	62,05 mg
Natriumstärkeglykolat	2,0 mg
Magnesiumstearat	1,0 mg
Gesamt	100,26 mg

Herstellungsverfahren

- Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Amlodipinmaleat, Magnesiumoxid und etwa 30% der Menge der mikrokristallinen Cellulose (MCC) wurden in einem Freifallmischer für 10 Minuten bei etwa 25 U/min vermischt.
- Die restliche Menge an MCC, wasserfreies Calciumhydrogenphosphat und Natriumstärkeglykolat wurden hinzugefügt und die Mischung wurde in einem Freifallmischer für 15

Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.

- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- 2,5 mg-Tabletten und proportional größere 10 mg-Tabletten wurden verpreßt, indem eine Korsch EK0-Exzenterpresse verwendet wurde.

Beispiel 5 Stabilitätsstudien an Amlodipinmaleat-Tabletten

Die Stabilitätsstudien an den Chargen, die in den Beispielen 1-4 hergestellt wurden, wurden in einer thermostatierten Kammer, eingestellt auf 40 +/- 2°C und 75 +/- 5% an relativer Feuchte, in verschiedenen Verpackungsmaterialien (HDPE-Flaschen, PVC/PVDC/PE-Blister) oder in einer offenen Schale durchgeführt. Die Bestimmung der wirksamen Substanz und des Gehaltes an Verunreinigungen wurde mittels einer HPLC-Methode durchgeführt, indem Referenzmaterialien des Amlodipinmaleats und der Hauptabbau-Verunreinigungen benutzt wurden:

Amlodipinaspartat (Z#204)
Amlo-Pyridin (Z#202)

Abgesehen davon wurden zwei geringfügigere Verunreinigungen Z#203 und Z#205 detektiert und identifiziert.

Der Gehalt an anderen detektierten Verunreinigungen/Abbauprodukten wurde durch interne Normierung berechnet.

In den folgenden Tabellen ist der Gehalt der wirksamen Substanz in Milligramm angegeben, der Gehalt an Verunreinigungen ist in Prozent angegeben.

A) Stabilitätsstudien, durchgeführt bei 40°C / 75% RF, offene Schale (Einfluß des pH-Werts)

t=0 Monate	(A)	(B)	(E)	(F)	(C)	(D)
Gehalt (mg/tab)	2,45	9,85	2,38	10,01	2,38	9,88
Z#202 (%)	0,15	0,15	0,08	0,08	0,05	0,08
Z#203 (%)	0,00	0,03	0,03	0,03	0,03	0,03
Z#204 (%)	0,16	0,15	0,13	0,12	0,13	0,12
Z#205 (%)	0,01	0,01	0,00	0,00	0,00	0,00
Gesamt unbekannt (%)	0,31	0,28	0,27	0,28	0,27	0,27
T= 1 Monat						
Gehalt (mg/tab)	2,48	9,82	2,31	9,55	2,23	9,29
Z#202 (%)	0,15	0,15	0,21	0,23	1,27	1,25
Z#203 (%)	0,00	0,03	0,04	0,04	0,04	0,04
Z#204 (%)	0,24	0,23	0,41	0,30	0,27	0,22
Z#205 (%)	0,01	0,01	0,03	0,02	0,04	0,03
Gesamt unbekannt (%)	0,35	0,31	0,58	0,56	0,83	0,72
T= 3 Monate						
Gehalt (mg/tab)	2,40	9,69	2,30	9,57	2,16	8,94
Z#202 (%)	0,20	0,18	0,37	0,39	1,85	1,85
Z#203 (%)	0,03	0,03	0,04	0,05	0,04	0,04
Z#204 (%)	0,31	0,29	0,77	0,63	0,33	0,27
Z#205 (%)	0,01	0,01	0,05	0,04	0,03	0,02
Gesamt unbekannt (%)	0,42	0,35	1,11	1,10	1,37	1,10

Stabilitätsstudien, durchgeführt in PVC/PE/PVDC-Blistern bei 40°C / 75% RF

T=0 Monate	(A)	(B)	(J)	(K)
Gehalt	2,51	9,99	2,52	10,33
Z#202	0,11	0,11	0,20	0,20
Z#203	0,00	0,00	0,00	0,00
Z#204	0,15	0,14	0,00	0,00
Z#205	0,01	0,01	0,00	0,00
Gesamt unbekannt	0,31	0,31	0,46	0,49
T= 3 Monate				
Gehalt	2,49	9,49	2,49	9,99
Z#202	0,15	0,15	0,07	0,05
Z#203	0,00	0,00	0,00	0,03
Z#204	0,26	0,29	0,46	0,40
Z#205	0,01	0,01	0,02	0,02
Gesamt unbekannt	0,36	0,34	0,45	0,38
T= 6 Monate				
Gehalt	2,45	9,49	2,45	9,94
Z#202	0,23	0,21	0,17	0,11
Z#203	0,04	0,04	0,04	0,04
Z#204	0,46	0,39	0,64	0,54
Z#205	0,01	0,00		0,02
Gesamt unbekannt	0,57	0,54	0,64	0,51

Stabilitätsstudien, durchgeführt in offener Schale bei 40°C / 75% RF

t=0 Monate	(J)	(K)
Gehalt	2,49	10,18
Z#202	0,04	0,04
Z#203	0,02	0,03
Z#204	0,19	0,17
Z#205	0,01	0,01
Gesamt unbekannt	0,42	0,36
t= 1 Monat		
Gehalt	2,44	10,14
Z#202	0,08	0,07
Z#203	0,02	0,03
Z#204	0,34	0,36
Z#205	0,01	0,01
Geaamt unbekannt	0,45	0,42
t= 3 Monate		
Gehalt	2,41	9,72
Z#202	0,22	0,21
Z#203	0,04	0,04
Z#204	0,50	0,54
Z#205	0,03	0,03
Gesamt unbekannt	0,63	0,56

Stabilitätsstudien, durchgeführt in HDPE-Behältern bei 40°C / 75% RF

T=0 Monate	(A)	(B)	(J)	(K)	(L)
Gehalt	2,51	9,99	2,52	10,33	10,11
Z#202	0,11	0,11	0,20*	0,20*	0,19*
Z#203	0,00	0,00	0,00	0,00	0,00
Z#204	0,15	0,14	0,00*	0,00*	0,00*
Z#205	0,01	0,01	0,00	0,00	0,00
Gesamt unbekannt	0,31	0,31	0,46	0,49	0,40
T= 3 Monate					
Gehalt	2,52	9,84	2,51	10,26	8,69
Z#202	0,13	0,15	0,41*	0,37*	0,45*
Z#203	0,00	0,00	0,03	0,00	0,05
Z#204	0,27	0,23	0,00*	0,00*	0,00*
Z#205	0,03	0,03	0,07	0,05	0,01
Gesamt unbekannt	0,33	0,32	0,41	0,49	0,66
T= 6 Monate					
Gehalt	2,49	9,83	2,47	10,26	
Z#202	0,15	0,15	0,08	0,05	
Z#203	0,00	0,00	0,00	0,03	
Z#204	0,44	0,41	0,44	0,38	
Z#205	0,02	0,01	0,10	0,08	
Gesamt unbekannt	0,46	0,44	0,44	0,37	

* Werte für Z#202 und Z#204 zusammen

Vergleichsstabilitätsstudien, durchgeführt mit der Zusammensetzung mit alkalischem pH-Wert (offene Schale, 40°C / 75% RF)

t=0 Monate	(X)
Gehalt	2,52
Z#202	0,04
Z#203	0,03
Z#204	0,13
Z#205	0,00
Gesamt unbekannt	0,30
t=1 Monat	
Gehalt	2,43
Z#202	0,06
Z#203	0,03
Z#204	1,73
Z#205	0,00
Gesamt unbekannt	0,51

Stabilitätsstudien, durchgeführt bei 40°C / 75% RF, offene Schale (Einfluß der Partikelgröße)

t = 0 Monat	(A)	(B)	(G)	(H)	(I)
Gehalt (mg/Tab)	2,45	9,85	2,38	10,12	2,54
Z#202 (%)	0,15	0,15	0,05	0,05	0,10
Z#203 (%)	0,00	0,03	0,00	0,01	0,04
Z#204 (%)	0,16	0,15	0,03	0,03	0,01
Z#205 (%)	0,01	0,01	0,00	0,00	0,02
Gesamt unbekannt (%)	0,31	0,28	0,17	0,11	0,26
t = 1 Monat					
Gehalt (mg/Tab)	2,48	9,82	2,29	9,88	-
Z#202 (%)	0,15	0,15	0,17	0,14	-
Z#203 (%)	0,00	0,03	0,01	0,01	-
Z#204 (%)	0,24	0,23	0,52	0,48	-
Z#205 (%)	0,01	0,01	0,02	0,02	-
Gesamt unbekannt (%)	0,35	0,31	0,22	0,19	-
t = 2 Monate					
Gehalt (mg/Tab)	2,46	9,87	2,22	9,64	2,35
Z#202 (%)	0,17	0,16	0,28	0,25	0,34
Z#203 (%)	0,00	0,00	0,01	0,01	0,03
Z#204 (%)	0,28	0,28	1,02	1,01	0,41
Z#205 (%)	0,01	0,01	0,03	0,03	0,04
Gesamt unbekannt (%)	0,47	0,43	0,63	0,52	0,60
t = 3 Monate					
Gehalt (mg/Tab)	2,40	9,69	2,24	9,36	2,36
Z#202 (%)	0,20	0,18	0,18	0,16	0,39
Z#203 (%)	0,03	0,03	0,01	0,01	0,11
Z#204 (%)	0,31	0,29	1,26	1,34	0,53
Z#205 (%)	0,01	0,01	0,05	0,04	0,04
Gesamt unbekannt (%)	0,42	0,35	0,90	0,74	0,64

Für einen Vergleich der Stabilität wird die Zunahme an Gesamt-Verunreinigungen, identifiziert und nicht identifiziert, bezüglich des Wertes bei t=0 Monaten betrachtet:

Zunahme an Gesamt-Verunreinigungen in %	(A)	(B)	(G)	(H)	(I)
Nach 3 Monaten bei 40°C / 75% RF	+ 0,33	+ 0,24	+ 1,82	+ 1,43	

Vergleichsstabilitätsstudien mit Norvasc (kommerzielle Amlodipinbesylat-Tabletten)

Stabilitätsstudien, durchgeführt in Originalblistern bei 40°C / 75% RF

	Norvasc® 5 mg Charge 81040100 (DE)	Norvasc® 10 mg Charge 901-05941 (NL)
t = 0 Monate		
Gehalt	5,19	9,99
Z#202	0,04	0,02
Z#203	0,01	0,00
Z#204	0,00	0,00
Z#205	0,00	0,00
Gesamt unbekannt	0,12	0,12
t = 3 Monate		
Gehalt	5,13	9,70
Z#202	0,16	0,17
Z#203	0,03	0,00
Z#204	0,00	0,00
Z#205	0,00	0,00
Gesamt unbekannt	0,38	0,62
t = 6 Monate		
Gehalt	4,97	9,58
Z#202	0,28	0,27
Z#203	0,00	0,00
Z#204	0,00	0,00
Z#205	0,00	0,00
Gesamt unbekannt	0,49	0,78

Stabilitätsstudien, durchgeführt in offener Schale bei 40°C / 75% RF

		Norvasc® 2,5 mg Chargen-Nummer 8QP115A (US)	Norvasc® 10 mg Chargen-Nummer N-09 (ES)
t = 0 Monate	Gehalt	2,44	9,91
	Z#202	0,08	0,82
	Z#203	0,00	0,00
	Z#204	0,00	0,00
	Z#205	0,00	0,00
	Gesamt unbekannt	0,02	0,34
t = 1 Monat	Gehalt	2,44	8,90
	Z#202	0,18	2,17
	Z#203	0,04	0,19
	Z#204	0,00	0,01
	Z#205	0,00	0,00
	Gesamt unbekannt	0,10	1,21
t = 2 Monate	Gehalt	2,39	7,98
	Z#202	0,27	3,24
	Z#203	0,00	0,00
	Z#204	0,00	0,00
	Z#205	0,00	0,00
	Gesamt unbekannt	0,33	2,51
t = 3 Monate	Gehalt	2,34	7,68
	Z#202	0,37	3,98
	Z#203	0,00	0,00
	Z#204	0,00	0,00
	Z#205	0,00	0,03
	Gesamt unbekannt	0,27	2,76

Beispiel 6 Amlodipinmaleat-Kapseln

Zusammensetzungen

Chargen-Nummer	(CA), (CB)
Äquivalent an Amlodipin	5,0 mg
Amlodipinmaleat	6,42 mg
Mikrokristalline Cellulose	72,6 mg
Vorgetrocknete Kartoffel-Stärke	20,0 mg
Magnesiumstearat	0,5 mg
Gesamt	99,52 mg

Chargen-Nummer	(CC)	(CX)
Äquivalent an Amlodipin	5,0 mg	5,0 mg
Amoldipinmaleat	6,42 mg	6,42 mg
Wasserfreies Calciumhydrogenphosphat	31,5 mg	31,5 mg
Magnesiumoxid	-	1,5 mg
Mikrokristalline Cellulose	62,0 mg	62,0 mg
Natriumstärkeglykolat	2,0 mg	2,0 mg
Magnesiumstearat	1,0 mg	1,0 mg
Gesamt	102,92 mg	104,43 mg

pH-Wert der Kapseln bei t=0

Chargen-Nr.	Gehalt	Typ von wasserfreiem Calciumhydrogenphosphat	pH-Wert der 20% (m/V)-Aufschlämmung des Kapselinhalts
(CC)	5,0 mg	Di CAFOS A	6,10
(CX)	5,0 mg	Di CAFOS A	9,59

Charge (CA) wurde wie folgt hergestellt:

- Das Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Alle Hilfsstoffe außer Magnesiumstearat wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.

- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- *Gelatine*kapseln wurden mit dieser Pulver-Mischung befüllt.

Charge (CB) wurde wie folgt hergestellt:

- Das Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Alle Hilfsstoffe außer Magnesiumstearat wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- *HPMC*-Kapseln wurden mit dieser Pulver-Mischung befüllt.

Charge (CC) wurde wie folgt hergestellt:

- Das Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Alle Hilfsstoffe außer Magnesiumstearat wurden in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- *Gelatine*kapseln wurden mit dieser Pulver-Mischung befüllt.

Charge (CX) wurde wie folgt hergestellt:

- Das Amlodipinmaleat wurde durch ein 500 µm-Sieb gesiebt.
- Die anderen Hilfsstoffe wurden durch ein 850 µm-Sieb gesiebt.
- Amlodipinmaleat, Magnesiumoxid und etwa 30% der Menge an mikrokristalliner Cellulose (MCC) wurden in einem Freifallmischer für 10 Minuten bei etwa 25 U/min vermischt.

- Die restliche Menge an MCC, wasserfreies Calciumhydrogenphosphat und Natriumstärkeglykolat wurden hinzugefügt und die Mischung wurde in einem Freifallmischer für 15 Minuten bei etwa 25 U/min vermischt. Der pH-Wert wurde bei einer 20%-igen wäßrigen Aufschlämmung kontrolliert.
- Magnesiumstearat wurde hinzugefügt und die Pulver-Mischung wurde für weitere 5 Minuten bei etwa 25 U/min vermischt.
- *Gelatine*kapseln wurde mit dieser Pulver-Mischung befüllt, indem eine automatische Kapsel-Füllmaschine verwendet wurde.

Beispiel 7 Stabilitätsstudien an Amlodipinmaleat-Kapseln

Die Stabilitätsstudien an Chargen, die in Beispiel 6 hergestellt wurden, wurden grundsätzlich wie in Beispiel 5 beschrieben durchgeführt.

Stabilitätsstudien, durchgeführt in PVC/PE/PVDC-Blistern bei 40°C / 75% RF

t=0 Monate	(CA)	(CB)
Gehalt	5,13	4,98
Z#202	0,04	0,04
Z#203	0,03	0,03
Z#204	0,12	0,12
Z#205	0,00	0,00
Gesamt unbekannt	0,32	0,31
t=3 Monate		
Gehalt	4,82	4,76
Z#202	0,08	0,06
Z#203	0,02	0,02
Z#204	0,15	0,14
Z#205	0,01	0,00
Gesamt unbekannt	0,39	0,38
t=6 Monate		
Gehalt	4,67	4,81
Z#202	0,14	0,10
Z#203	0,04	0,02
Z#204	0,23	0,18
Z#205	0,02	0,01
Gesamt unbekannt	0,45	0,42

Zwei Ein-Monats-Stabilitätsstudien, durchgeführt in offener Schale

t=0 Monate	(CC)	(CX)
Gehalt	4,91	4,72
Z#202	0,04	0,04
Z#203	0,03	0,04
Z#204	0,12	0,13
Z#205	0,00	0,00
Gesamt unbekannt	0,28	0,29
T=1 Monat bei 25°C/60% RF		
Gehalt	4,85	4,70
Z#202	0,05	0,05
Z#203	0,04	0,04
Z#204	0,13	0,14
Z#205	0,00	0,00
Gesamt unbekannt	0,28	0,28
T=1 Monat bei 40°C/75% RF		
Gehalt	4,76	4,16
Z#202	0,06	0,08
Z#203	0,04	0,03
Z#204	0,15	11,36
Z#205	0,00	0,00
Gesamt unbekannt	0,28	0,62

Stabilitätsstudien, durchgeführt in HDPE-Behältern

t=0 Monate	(CA)	(CB)
Gehalt	4,99	5,01
Z#202	0,05	0,04
Z#203	0,03	0,03
Z#204	0,12	0,11
Z#205	0,00	0,00
Gesamt unbekannt	0,31	0,30
T = 3 Monate bei 25°C/60% RF		
Gehalt	5,07	4,77
Z#202	0,04	0,04
Z#203	0,03	0,04
Z#204	0,12	0,12
Z#205	0,00	0,00
Gesamt unbekannt	0,30	0,32
T=3 Monate bei 40°C/75% RF		
Gehalt	5,01	4,65
Z#202	0,05	0,05
Z#203	0,03	0,04
Z#204	0,12	0,13
Z#205	0,00	0,00
Gesamt unbekannt	0,29	0,32

Stabilitätsstudien an kommerziell erhältlichen Amlor® (Amlodipinbesylat)-Kapseln

Stabilitätsstudien durchgeführt im Originalblister

Amlor 5 mg Kapseln Charge 9037002	t=0 Monate	t=3 Monate 40°C/75% RF
Gehalt	4,59	4,44
Z#202	0,01	0,20
Z#203	0,00	0,00
Z#204	0,00	0,00
Z#205	0,00	0,00
Gesamt unbekannt	0,06	0,37

Schutzansprüche

1. Pharmazeutische Zusammensetzung, umfassend eine wirksame Menge an Amlodipinmaleat und mindestens einen pharmazeutisch annehmbaren Hilfsstoff, wobei die Zusammensetzung einen pH-Wert innerhalb eines Bereich von 5,5-7,0 hat.

2. Zusammensetzung gemäß Anspruch 1, wobei die Zusammensetzung einen pH-Wert von etwa 6,0-7,0 hat.

3. Zusammensetzung gemäß Anspruch 1 oder 2, wobei die Zusammensetzung in fester Form vorliegt.

4. Zusammensetzung gemäß einem der vorherigen Ansprüche, wobei der Hilfsstoff Calciumphosphat oder mikrokristalline Cellulose ist.

5. Zusammensetzung gemäß Anspruch 4, wobei die Zusammensetzung Calciumphosphat und mikrokristalline Cellulose umfaßt.

6. Zusammensetzung gemäß Anspruch 4, wobei der Hilfsstoff Calciumhydrogenphosphat ist.

7. Zusammensetzung gemäß Anspruch 4, wobei der Hilfsstoff mikrokristalline Cellulose ist.

8. Zusammensetzung gemäß einem der vorstehenden Ansprüche, des weiteren ein saures pH-Einstellmittel umfassend.

9. Zusammensetzung gemäß einem der vorstehenden Ansprüche, wobei die Zusammensetzung di Form einer Tablette hat.

10. Zusammensetzung gemäß Anspruch 9, wobei die Tablette des weiteren eine Außenfeuchtigkeits- und/oder Lichtsperrschicht umfaßt.

11. Zusammensetzung gemäß einem der vorstehenden Ansprüche1-8, wobei die Zusammensetzung die Form von einer Kapsel hat.

12. Zusammensetzung gemäß einem der vorstehenden Ansprüche, wobei die Menge an Amlodipinmaleat 1,0 bis 25 mg an freier Amlodipinbase entspricht.

13. Zusammensetzung gemäß Anspruch 12, wobei die Menge an Amlodipinmaleat 1,25, 2,5 , 5 oder 10 mg an freier Amlodipinbase entspricht.

14. Zusammensetzung gemäß einem der vorstehenden Ansprüche, herstellbar durch ein Verfahren, bei dem Amlodipinmaleat und mindestens ein pharmazeutisch annehmbarer Hilfsstoff zu einer Mischung vermischt werden, die einen pH-Wert innerhalb eines Bereiches von 5,5 bis 7 hat.

15. Zusammensetzung gemäß Anspruch 14, wobei die Mischung zu einer Tablette verpreßt wird.

16. Zusammensetzung gemäß Anspruch 14, wobei die Mischung in eine Kapsel abgefüllt wird.

17. Zusammensetzung gemäß Anspruch 14, wobei das Vermischen mittels Feuchtgranulation durchgeführt wird.

18. Zusammensetzung gemäß Anspruch 14, wobei das Vermischen mittels eines trockenen Verfahrens durchgeführt wird.

19. Zusammensetzung gemäß Anspruch 18, wobei das Amlodipinmaleat in Form fester Partikel vermischt wird, wobei diese Partikel eine durchschnittliche Größe von mindestens 100 µm haben.

20. Zusammensetzung gemäß Anspruch 19, wobei die durchschnittliche Partikelgröße des Amlodipinmaleats mindestens 20 µm beträgt.

AST 1

US005155120A

United States Patent [19]

Lazar et al.

[11] Patent Number: 5,155,120

[45] Date of Patent: Oct. 13, 1992

[54] METHOD FOR TREATING CONGESTIVE HEART FAILURE

[75] Inventors: Jeffrey D. Lazar; Joseph F. Soulsrada; Svetislav K. Vanov, all of Groton, Conn.

[73] Assignee: Pfizer Inc, New York, N.Y.

[21] Appl. No.: 767,173

[22] Filed: Sep. 27, 1991

Related U.S. Application Data

[63] Continuation of Ser. No. 650,838, Jan. 14, 1991, abandoned.

[51] Int. Cl.[5] A01N 43/40

[52] U.S. Cl. 514/356

[58] Field of Search 514/356

[56] References Cited

PUBLICATIONS

Dunselman et al, "Efficacy of Felodipine in Congestive Heart Failure," Eur; Heart J. 10:354–364 1989.

Packer, "Calcium Channel Blockers in Chronic Heart Failure" Circulation 82:2254–2257 1990.

Primary Examiner—Frederick E. Waddell

Assistant Examiner—Gregory Hook

Attorney, Agent, or Firm—Peter C. Richardson; J. Trevor Lumb; James M. McManus

[57] ABSTRACT

Method for the treatment of congestive heart failure using amlodipine and the pharmaceutically acceptable acid addition salts thereof.

3 Claims, No Drawings

METHOD FOR TREATING CONGESTIVE HEART FAILURE

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of copending application Ser. No. 07/650,838, filed Jan. 14, 1991, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to the antihypertensive agent amlodipine and its pharmaceutically acceptable acid addition salts and their use in treating congestive heart failure.

2. Description of the Prior Art

Congestive heart failure, regardless of its etiology, is characterized by a weakness of the myocardial tissue of the left and/or right ventricles of the heart to pump and circulate blood into systemic and/or pulmonary circulations. It is accompanied by circulatory and neurohumoral changes which result in failure to deliver sufficient blood and oxygen supply to peripheral tissues and vital organs. If left untreated the health of a patient with congestive heart failure could deteriorate to the point where the disease would be fatal.

While there are several therapies available for the treatment of congestive heart failure, the most widely used is digitalis. Its use is limited because of its slow onset of action and the small difference between the maximum therapeutic and minimum toxic dose levels.

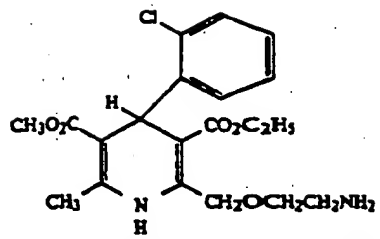
SUMMARY OF THE INVENTION

A therapy has now been found for treating congestive heart failure in a human subject having such condition which comprises orally or parenterally administering to said human subject a congestive heart failure treating amount of amlodipine and the pharmaceutically acceptable acid addition salts thereof. It is preferred that the daily dosage be given in unit dosage form of 10 mg once a day orally.

While amlodipine belongs to a family of calcium channel blockers useful as antihypertensive and in the treatment of ischemic heart disease such agents are considered ineffective in treating congestive heart disease and may be deleterious if administered to a patient in heart failure. Consequently, the discovery that amlodipine is useful in treating congestive heart failure is unexpected.

DETAILED DESCRIPTION OF THE INVENTION

As previously mentioned, the antihypertensive compound of the present method invention is known in the art. Amlodipine, 3-ethyl-5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate, and its pharmaceutically acceptable acid addition salts are claimed and their preparation described in U.S. Pat. Nos. 4,572,909 and 4,879,303. The chemical structure of amlodipine is as follows:



I

p Although the generic name of amlodipine represents the free base, the present method invention is meant to embrace pharmaceutically acceptable acid addition salts, such as hydrochloride, hydrobromide, hydroiodide, nitrate, sulfate, bisulfate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, gluconate, methanesulfate, ethanesulfate, benzenesulfonate and p-toluenesulfonate salts.

In the treatment of congestive heart failure it is generally preferred to administer amlodipine or its pharmaceutically acceptable acid addition salts orally once a day. In using amlodipine or one of its pharmaceutically acceptable acid addition salts in congestive heart failure, a dosage level of 5 mg to 20 mg per day is therapeutically effective, and a preferred dose is 10 mg per day.

It is to be appreciated that still other variations may also occur in this respect, depending upon the individual response to said medicament, as well as on the particular type of pharmaceutical formulation chosen and the time period and interval at which such administration is carried out. In some instances, dosage levels below the lower limit of the aforesaid range may be more than adequate, while in other cases still larger dosages may be employed without causing any harmful or deleterious side effects to occur provided that such higher dose levels are first divided into several smaller doses that are to be administered throughout the day. Amlodipine can be given alone or in combination with digitalis, thiazide diuretics, other diuretics, ACE inhibitors, etc.

For purposes of oral administration, tablets containing various excipients such as sodium citrate, calcium carbonate and dicalcium phosphate may be employed along with various disintegrants such as starch and preferably potato or tapioca starch, alginic acid and certain complex silicates, together with binding agents such as polyvinylpyrrolidine, sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, sodium lauryl sulfate and talc are often very useful for tableting purposes. Solid compositions of a similar type may also be employed as fillers in soft elastic and hard-filled gelatin capsules; preferred materials in this connection also include lactose or milk sugar as well as high molecular weight polyethylene glycols. When aqueous suspension and/or elixirs are desired for oral administration, the essential active ingredient may be combined with various sweetening or flavoring agents, col ring matter or dyes and, if so desired, emulsifying and/or suspending agents, together with such diluents as water, ethanol, propylene glycol, glycerin and various like combinations thereof.

Although the preferred mode of administration of amlodipine or one of its pharmaceutically acceptable acid addition salts is oral, they may be administered parenterally as well.

For purposes of parenteral administration, solutions of amlodipine in sesame or peanut oil or in aqueous-propylene glycol may be employed, as well as sterile aqueous solutions of the corresponding water-soluble acid addition salts previously enumerated. Such aqueous solutions should be suitably buffered if necessary and the liquid diluent rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular and subcutaneous injection purposes. In this connection, the sterile aqueous media employed are readily obtained by standard techniques well known to those skilled in the art. For instance, distilled water is ordinarily used as the liquid diluent and the final preparation is passed through a suitable bacterial filter, such as a sintered-glass filter or a diatomaceous-earth or unglazed porcelain filter. Preferred filters of this type include the Berkefeld, the Chamberland and the asbestos disc-metal Seitz filter, wherein the fluid is sucked through the filter candle into a sterile container with the aid of a suction pump. The necessary steps should be taken throughout the preparation of these injectable solutions to ensure that the final products are obtained in a sterile condition.

The following examples are provided solely for the purpose of illustration and are not to be construed as limitations of this invention, many variations of which are possible without departing from the spirit or scope thereof. The clinical studies which comprise the following example were conducted with amlodipine benzenesulfonate.

EXAMPLE

I. Purpose of Study

The purpose of the study was to evaluate the efficacy of fixed doses amlodipine tablets (10 mg/day) compared with placebo in the chronic treatment (8 wks) of patients with significantly compromised left ventricular function.

II. Trial Design

This was a randomized, parallel, double-blind, placebo-controlled study of amlodipine in outpatients with NYHA functional class IIM-III chronic heart failure. The duration of this study was 10 weeks; a 2 week single-blind placebo phase was followed by an 8 week double-blind phase during which patients received either placebo or amlodipine. The study consisted of three phases:

(1) stabilization and screening phase.
(2) single-blind phase.
(3) double-blind phase.

Stabilization and Screening Phase

Prior to entry into the study, the patient had to meet the following criteria:

1) patient body weight had to be stable (±4 pounds) for at least two weeks;

2) in patients on ACE inhibitors, therapeutic regimen had to be constant for 12 weeks;

3) therapeutic regimens with digitalis and diuretics had to be constant for at least four weeks;

4) therapy which was specifically listed in exclusion criteria had to be discontinued for at least two weeks.

Upon meeting these criteria, patients underwent baseline screening which included clinical evaluation, one treadmill exercise test, a chest X-ray, laboratory safety tests, and determination of left ventricular ejection fraction. In several centers, patients had radionuclide angiographic studies using the multiple gated equilibrium cardiac blood-pool imaging technique (at rest and optionally on exercise). The use of two dimensional and Doppler echocardiography was optional. Once the screening visit was completed, patients entered the single-blind phase of study.

Single-blind Outpatient Phase

During the single-blind outpatient phase, all patients had to complete 2 consecutive treadmill tests with total exercise time within 15% of one another. One additional exercise test was allowed if the duration of the two previous tests were not within 15%. However, in order to qualify for continuation in this study, exercise tests took place within the time period of 14-28 days (single-blind placebo phase). After the last exercise test was completed, 24-hour Holter ECG monitoring was performed on those patients who qualified for randomization.

Double-blind Outpatient Phase

After the single-blind phase, patients who qualified were randomized into the double-blind outpatient phase (8 week duration). Patients were allocated to treatments by means of computer generated randomized lists. The assignment was stratified by the presence or absence of ACE inhibitor therapy. Drug was provided for the two groups identified as "ACE Inhibitors"; and "No ACE Inhibitors," respectively.

The patients were evaluated at the end of study weeks 4, 6, 8 and 10 (2, 4, 6, and 8 weeks of double-blind treatment). At each clinic visit, a sitting blood pressure, pulse rate, and body weight was determined. A 12-lead ECG, treadmill exercise test, and a 24 hour Holter ECG monitoring was performed at the end of study weeks 6 and 10. Radionuclide angiography, two dimensional, and Doppler echocardiography (these two tests were optional and were done in selected centers), and chest X-ray were performed at the end of week 10.

Additional Optional Parameters

At the end of weeks 2 and 10 of the study, 50 ml of blood was collected for optional determination of cardiorenal hormones. Determination of gated radionuclide ventriculography at rest and during exercises was done at screening and at the end of week 10.

III. Subject Selection Criteria

A. Inclusion in Study

1. Age 21-80 years.
2. Males, or non-pregnant, non-lactating or postmenopausal females.
3 Outpatients.
4 Medical history and physical examination before inclusion in the study.
5. Written informed consent from the patient to participate in the study.
6. Diagnostic criteria, each of the following:
   a) clinical diagnosis of congestive heart failure.
   b) objective evidence of CHF, present for at least 6 weeks detected by: left ventricular ejection fraction of 0.40 or less at rest (as measured by radionuclide angiography or contrast ventriculography) and,
   c) New York Heart Association functional class IIM to III.
7. Etiology f heart failure to be:
   a) Ischemic heart disease,
   b) Myocardial disease of known or unknown cause.

8. All patients to be in stable clinical condition and could receive concomitant therapy with furosemide and a digitalis preparation, ACE inhibitors and/or vasodilators such as intermittent therapy with sublingual nitroglycerine. However, ACE inhibitor dosages must have been constant for at least 12 weeks. Sublingual nitroglycerin p.r.n. was allowed for angina attack relief.
9. Patients must be capable of undertaking a treadmill exercise test using the modified Naughton protocol and have a duration of at least 2 minutes but less than 16 minutes.

IV. Clinical Observation and Laboratory Measurements

A. Efficacy

Effect of amlodipine treatment on efficacy parameters was evaluated as follows:

1. Symptom Rating: At each visit patients received a detailed cardiopulmonary assessment of symptoms (dyspnea, fatigue, orthopnea, paroxysmal nocturnal and clinical dyspnea and edema) signs including lung congestion and ankle and pedal edema graded on a 4 point scale in absolute terms.
2. Patient Self-Assessment (Quality of Life Questionnaire): At each review visit, patients completed the self-assessment analysis of symptoms questionnaire (graded on a "thermometer scale" and exercise capabilities duration of a fixed pattern of activity) recorded at screening, at the end of single-blind period, after 4 weeks of double-blind therapy and at the end of double-blind therapy.
3. Global Evaluation: The investigator determined a global graded estimate of the patient's condition at each review visit and at the end of the double-blind therapy.
4. The following parameters were measured at baseline, at the end of 2 weeks single-blind therapy, after 4 weeks of double-blind therapy, and at the end of 8 weeks of double-blind therapy.
   a) 24-hour Holter monitoring;
   b) Standard 12-lead ECG immediately prior to each exercise test;
   c) Exercise tolerance (duration) as measured by treadmill exercise testing using the modified Naughton protocol. Optional measurement of oxygen consumption at peak exercise was also included.
   d) In selected centers, resting radionuclide imaging left ventricular ejection fraction was performed at screening and at the end of the double-blind phase (10th week of study). There was an optional determination of two-dimensional echocardiography and Doppler echocardiography and measurement of cardiorenal hormones in selected centers.

V. Statistical Methodology

Baseline for total exercise time was defined as the mean recorded time (in secs) of the last 2 exercise tests during the single-blind placebo phase. The baseline LVEF was at the screening visit. Baseline for ECG, body weight and norepinephrine was at week 2.

The statistical analyses for efficacy parameters were carried out on two subsets of patients corresponding to two definitions of study "end";

1) Final-baseline Analysis: All patients who completed the study are included in this subset. For the total exercise time, patients also have to satisfy the "reproducible" criterion for their exercise test. That is, the two consecutive treadmill tests have to be within 120 to 900 seconds and within 15% f one another.

2) Intent to Treat Analysis: All patients who had efficacy measurements collected during the double-blind treatment period were included for this analysis.

The comparison between treatment groups for the above mentioned parameters were based on the difference in the log (base e) scale; i.e., the log change is defined as:

$$c = ln(Final\ Test) - ln(Baseline\ Test)$$

which can also be expressed as

$$c = ln(Final/Baseline).$$

That is, c is the log-ratio of the parameter between the final and baseline visits.

The mean percentage change between the final and baseline visit can be obtained through anti-log transformation as:

$$\%\ change = [exp(c) - 1]*100$$

where

$$c = mean\ [log(Final) - log(Baseline)]$$

The statistical assessments of between treatment group difference in changes from baseline to final visit for the total exercise time, LVEF, norepinephrine, body weight and ECG parameters were performed by a three-way analysis of variance (ANOVA):

$$Change_{ijkl} = M + T_i + C_j + B_k + B*T_{ik} + E_{ijkl}$$

where:

$Change_{ijkl}$ is the change for the lth patient with the ith treatment and the kth background ACE inhibitor at center j

M is the overall mean

T_i is the ith treatment group, i=1(amlodipine), 2(placebo)

C_j is the fixed center effect, j=1 to 14

B_k is the background ACE inhibitor therapy, k=(ACE, 2(No ACE)

$B*T_{ik}$ is the treatment and background ACE inhibitor therapy interaction term

E_{ijkl} represents the random error and E is normally distributed N(0,d).

The statistical assessments of between treatment group difference in changes from baseline to final visit for each background therapy groups were performed by a two-way analysis of variance (ANOVA):

$$Change_{ijk} = M + T_i + C_j + E_{ijk}$$

where:

$Change_{ijk}$ is the change for the kth patient with ith treatment at center j

M is the overall mean

T_i is the ith treatment group, i=1(amlodipine), 2(placebo)

C_j is the fixed center effect, j=1 to 14

E_{ijk} represents the random error and E is normally distributed N(0,d).

Treatment effects were based on the ANOVA model using Type III Sum of Squares to derive F-statistics and Least Squares Estimates (LSE). A 95% confidence interval was derived from the LSE which had a t-distribution with degrees of freedom equal to those of the error Sums of Squares of the ANOVA model. Two-tailed tests were performed on all parameters.

To compare the average effect between amlodipine and the effect of placebo, a Least Square Estimate (LSE) based on the above above three-way ANOVA model was used. The LSE for this difference was obtained by the ESTIMATE option in SAS GLM procedure.

The SAS procedure FREQ with the RANK option was used to perform a Cochran-Mantel-Hanszel test for the investigator's global evaluation, and endpoint to baseline change in NYHA class, dyspnea, nocturnal dyspnea, orthopnea, fatigue and edema.

VI. Baseline Patient Characteristics

A total of 19 female and 99 male patients participated in this study. Both treatment groups were balanced with respect to age, body weight, race, and NYHA class (i.e., Class II and III). Duration of CHF in the amlodipine group was 34 months, and 26 months in the placebo group.

About half of the patients in each group had a history of old myocardial infarction and/or angina pectoris, and symptoms of chronic ischemic heart disease. A significant number also had conduction disorders of the heart and diabetes mellitus. As a whole, the two groups of patients were comparable in terms of the profile and severity of baseline disease.

The majority of patients were on cardiac glycosides (amlodipine 48, placebo 51) and loop diuretics (amlodipine 44, placebo 50). There was no clinically important changes in the use of or dosages of these drugs during the study. Out of 118 patients, 79 (38 amlodipine, 41 placebo) patients were also taking converting enzyme inhibitors. The two treatment groups were also well balanced within the subsets of patients receiving or not receiving ACE inhibitors.

VII. Drug Administration

Medication was dispensed in identically matching amlodipine and placebo tablets with individually coded bottles prepared for each patient.

The patients received amlodipine or placebo in a single tablet q.d. Double-blind medication was taken in the morning for a period of 8 weeks. Clinical deterioration, with objective evidence of fluid retention, was treated by an increase in furosemide dosage or other diuretics as needed.

Patients were instructed to take 1 tablet each morning except on the morning of a study visit; patients came to the visit undosed. After all study parameters were completed the study medication was dispensed from a new bottle.

The duration (range) of treatment for amlodipine was 50.2 (10–71) days and 50.9 (5–74) days for placebo.

VIII. Results

A. Efficacy

Exercise Performance: Exercise performance was one of the primary end points to be analyzed in this study. Prior to randomization, potential subjects were required to participate in two exercise tests and the total exercise time had to be within 15% of one another. All exercise tests were to be conducted at same time of the day, 0.5–4 hours after dosing and at least 2 hours after a light meal. Termination of the exercise was based only on dyspnea and/or fatigue (not chest pain or ST segment depression). Exercise time was assessed three times prior to randomization and after 4 and 8 weeks of double-blind treatment.

After 8 weeks of therapy, there was a greater increase in exercise time in the amlodipine group than in the placebo group. This increase was statistically significant in both evaluable patient analysis ($p=0.02$) and intent to treat analysis ($p=0.019$). In the amlodipine intent-to-treat group, the increase in exercise time was 74 seconds (14.5%) as compared to 17 seconds (3.0%) in the placebo group.

In the above analysis of exercise time, a comparison was based on the mean exercise time of two baseline exercise tests. To minimize a "learning" effect of exercise, the data was also analyzed using only the last baseline exercise test. After 8 weeks of therapy, there was a greater ($p=0.0354$) increase in exercise time in the amlodipine intent-to-treat group as compared to the placebo group.

In the case of clinical deterioration the protocol allowed an adjustment of diuretic dosing. Thus, it can be speculated that an improvement in exercise time after amlodipine was related to the increase in diuretic therapy. This possibility was examined but failed to establish the relationship between the increase in diuretic therapy and the improvement in exercise time.

Investigator's Rating: At the end of the study, the investigator's global assessment showed that more patients improved ($p<0.027$) on amlodipine (31/56, 55%) than on placebo (17/58, 29%).

The percent of improvement in the ACE inhibitor group was 57% (17/37) in amlodipine; 33% (13/39) in placebo. The percent of patients improving was smaller for the group with no ACE inhibitors: 53% (10/19) in amlodipine and 21% (4/19) in placebo.

Left Ventricular Ejection Fraction (LVEF): Treatment with amlodipine did not affect LVEF in the trial as a whole. However, LVEF tended to increase with amlodipine in patients treated with converting enzyme inhibitors, but not in the smaller group of patients who did not receive ACE inhibitors.

Plasma Norepinephrine: Plasma norepinephrine decreased in patients treated with amlodipine (358 to 263 pg/ml), but increased on placebo (365 to 439 pg/ml, amlodipine vs placebo, $p=0.018$). Similar quantitative changes were observed in the group receiving ACE inhibitors as well as in group not receiving ACE inhibitors.

Body Weight: At the end of the study, there were no significant differences in body weight between the amlodipine and placebo groups and amlodipine and placebo groups when analyzed by the presence of edema.

Peripheral Edema: Two percent (2%) of patients in the amlodipine group and 3% in the placebo group showed an improvement in peripheral edema. Seventy-four percent (74%) of patients in the amlodipine group and 84% of patients in the placebo group showed on change in peripheral edema. Twenty-four percent (24%) of the amlodipine treated patients and 13% of the placebo patients showed a deterioration of edema. These differences were not statistically significant.

Patients Clinical Symptoms: The table below summarizes the changes in NYHA class, dyspnea, nocturnal

dyspnea, and fatigue during therapy. In all of these indices, an improvement was detected with amlodipine treatment as compared to placebo. An improvement in nocturnal dyspnea and fatigue bserved after amlodipine therapy approached a statistical significance ($p=0.06$).

Parameters	Group	Number of Patients Who: Improved	Had No Change	Worsened	Total No. Pts.	P Value Baseline vs. 8 weeks
NYHA Class	Amlodipine	12	33	3	48	0.229
	Placebo	10	31	8	49	
Dyspnea	Amlodipine	15	28	9	52	0.318
	Placebo	13	29	15	57	
Nocturnal Dyspnea	Amlodipine	6	43	4	53	0.06
	Placebo	3	45	9	57	
Orthopnea	Amlodipine	6	43	4	53	0.205
	Placebo	10	45	1	56	
Fatigue	Amlodipine	23	23	7	53	0.06
	Placebo	15	29	12	56	

Patients Quality of Life Questionnaire: Patient self assessment was made on study weeks 2, 6 and 10 which consisted of 15 questions. These questions were converted into seven (7) CHF-QOL scales including dyspnea, fatigue, affect, locus of control, sleep, life satisfaction, and total evaluation of quality of life. The results indicate that treatment effects were in favor of amlodipine for those symptoms—specific scales most related to disease activity. Patients receiving amlodipine reported an improvement in dyspnea and fatigue. This improvement was statistically significant ($p<0.01$). All changes in quality of life assessment were in favor of amlodipine, and the total score p-value was equal to 0.06. The table below summarizes the key efficacy and safety parameters which showed an improvement or favorable trend in amlodipine treated (intent-to-treat analysis) patients as compared to the placebo group.

	Amlodipine	Placebo
Mean Increase in Exercise Time in sec: (N = 50 amlodipine, 54 placebo)	73.7 (14.5%)	17.3 (3.0%)
Investigators Global Assessment:		
Worsening	10	10
No Change	15	31
Improvement	31	17
Increase in LV Ejection Fraction (%): (N = 47 for amlodipine and placebo)	2.8 (11.5%)	1.1 (6.9%)

Conclusions

In this double-blind study, the clinical safety and efficacy of amlodipine was evaluated in 118 patients with heart failure. All patients had NYHA class II or III symptoms, left ventricular ejection fraction less than 40%, and the majority were treated with digoxin and diuretics. The analysis of data revealed that after 8 weeks of double-blind therapy, exercise time was significantly increased in the amlodipine treated group as compared to the placebo group. In addition, more patients treated with amlodipine experienced an improvement in CHF (NYHA class & related symptoms). Although amlodipine did not affect left ventricular ejection fraction, this particular variable tended to increase with amlodipine in patients treated with converting enzyme inhibitors. In patients treated with amlodipine, plasma norepinephrine significantly decreased, and significantly increased in the placebo group. Furthermore, as detected by a quality of life questionnaire, patients treated with amlodipine had a statistically significant improvement in both dyspnea and fatigue.

This study showed that in patients with class II–III CHF, amlodipine therapy increased exercise time, improved symptoms, and had no effect on ejection fraction in patients treated with or without ACE inhibitors.

We claim:

1. A method of treating congestive heart failure in a human subject having such condition which comprises orally administering to said human subject a congestive heart failure treating amount of amlodipine as a pharmaceutically acceptable acid addition salt thereof.

2. The method of claim 1, wherein the daily dosage is in unit dosage form of 10 mg.

3. The method of claim 2, wherein the daily dosage is given once a day.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,155,120

DATED : October 13, 1992

INVENTOR(S) : Jeffrey D. Lazar, et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 5, line 22 "nal and clinical dyspnea and edema) signs" should read –nal dyspnea and edema) and clinical signs–; and

Claim 1, column 10, line 45, "as" should read –or–.

Signed and Sealed this

Second Day of June, 1998

Attest:

Attesting Officer

BRUCE LEHMAN

Commissioner of Patents and Trademarks

AST 2

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 31/135, 31/275, 31/44, 31/445, 31/495, 31/55

A1

(11) International Publication Number: WO 95/34299

(43) International Publication Date: 21 December 1995 (21.12.95)

(21) International Application Number: PCT/US95/07526

(22) International Filing Date: 9 June 1995 (09.06.95)

(30) Priority Data:
08/259,080 10 June 1994 (10.06.94) US

(71) Applicant: TELOR OPHTHALMIC PHARMACEUTICALS, INC. [US/US]; 265 Ballardvale Road, Wilmington, MA 01887 (US).

(72) Inventors: BELMONTE-MARTINEZ, Carlos; Las Jacarandas, Calle Lamaignere, 2, Pl, 2ºB, E-03550 San Juan De Alicante (ES). NEUFELD, Arthur, H.; 100 Wildwood Road, Andover, MA 01810 (US).

(74) Agent: GATES, Edward, R.; Wolf, Greenfield & Sacks, P.C., 600 Atlantic Avenue, Boston, MA 02210 (US).

(81) Designated States: CA, JP, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: INHIBITION OF INTRAOPERATIVE MIOSIS/PRODUCTION OF MYDRIASIS BY CALCIUM CHANNEL BLOCKERS

(57) Abstract

This invention relates to a method for inhibiting intraoperative miosis or producing intraoperative mydriasis wherein a calcium channel blocker is introduced into an intraocular chamber of a subject undergoing intraocular surgery. Kits are provided for supplying the surgeon with an ophthalmologically-acceptable solution containing an effective amount of the calcium channel blocker.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo			SE	Sweden
CH	Switzerland	KR	Republic of Korea	SI	Slovenia
CI	Côte d'Ivoire	KZ	Kazakhstan	SK	Slovakia
CM	Cameroon	LI	Liechtenstein	SN	Senegal
CN	China	LK	Sri Lanka	TD	Chad
CS	Czechoslovakia	LU	Luxembourg	TG	Togo
CZ	Czech Republic	LV	Latvia	TJ	Tajikistan
DE	Germany	MC	Monaco	TT	Trinidad and Tobago
DK	Denmark	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	US	United States of America
FI	Finland	ML	Mali	UZ	Uzbekistan
FR	France	MN	Mongolia	VN	Viet Nam
GA	Gabon				

INHIBITION OF INTRAOPERATIVE MIOSIS /PRODUCTION OF MYDRIASIS BY CALCIUM CHANNEL BLOCKERS

Field of the Invention

This invention relates to a method for inhibiting intraoperative miosis or producing intraoperative mydriasis, wherein a calcium channel blocker is introduced into an intraocular chamber of a subject undergoing intraocular surgery. Kits are provided for supplying the surgeon with an ophthalmologically-acceptable solution containing an effective amount of the calcium channel blocker.

Background of the Invention

During intraocular surgery, particularly during the removal of the cataractous lens, a small pupil in the operative eye can impair the work of the ophthalmic surgeon. A smaller pupil generally is found in older subjects, those individuals most frequently undergoing intraocular surgery. Moreover, the pupil becomes constricted or miotic when the eye is opened for surgery. Manipulation of intraocular instruments and the lens material is difficult when the pupil is miotic.

In order to maintain a desirable operative field during intraocular surgery, constriction of the pupil should be prevented, inhibited or reversed or dilation of the pupil (mydriasis) should be accomplished. Mechanical devices for physically retracting the pupil during surgery have been proposed. See, e.g. U.S. Patents Nos. 4,991,567 and 4,782,820. Positioning of these devices, however, is time-consuming, and such devices are not suitable for many intraocular surgical procedures involving delicate movements of instruments and tissues.

Pharmacological agents have been sought for inhibiting miosis or producing mydriasis. Before surgery, topical non-steroidal anti-inflammatory agents have been applied to prevent intraoperative miosis, but this treatment is only minimally effective. (Keates et al Ann. Ophthalmol. 16(10) 919-921 (1984) It is known that the pupil dilates during retrobulbar anesthesia but that this dilation is subsequently lost. During surgery, epinephrine has been added to intraocular irrigating solutions, but this drug does not often reverse miosis or produce mydriasis to a significant extent. Lotti (U.S. patent no. 5,153,205) teaches topical application

of cholinergic M3 receptor antagonists to inhibit miosis. Nagy (U.S. patent no. 4,960,799) teaches topical administration of beta-blockers to treat inflammation of the ey and discloses that the beta-blockers also inhibit miosis during eye surgery. Bock et al. (U.S. patent no. 5,218,114) disclose that cholecystokinin antagonists may be used during intraocular surgery to prevent miosis.

The effectiveness of certain of the foregoing compounds for inhibiting miosis or producing mydriasis during intraocular surgery is not accepted by those skilled in the art. None are used universally.

Calcium channel blocking agents have been applied topically to the eye to reduce intraocular pressure and treat glaucoma. See, e.g., Abelson, U.S. patent no. 4,981,871. The use of an N-type and an L-type calcium channel blocker as an inhibitor of miosis also has been explored, but the results were not successful. (European Journal of Pharmacology, 209 (1991) 175-183). This study involved the use of δ-conotoxin, an N-type blocker, to block ruthenium red induced miosis. This toxin was successful in blocking the miosis induced by the ruthenium red; δ-conotoxin, however, is noxious and cannot be used therapeutically to treat miosis in the eye. Nifedipine, an L-type blocker, also was tested in this model, but was unable to block the miosis induced by the ruthenium red.

Summary of the Invention

It has been discovered that L-type calcium channel blockers inhibit miosis associated with intraocular surgery. This discovery is surprising in that an L-type calcium channel blocker was unable to block miosis associated with ruthenium red. As discussed in greater detail below, it is believed that ruthenium red acts through different pathways in causing miosis than the pathways responsible for miosis associated with intraocular surgery. The invention is believed to take advantage of this difference.

According to one aspect of the invention, a method for inhibiting intraoperative miosis or producing intraoperative mydriasis is provided. An effective amount of an L-type calcium channel blocker is introduced into an intraocular chamber of a subject, substantially simultaneously with performing intraocular surgery on the subject. Examples of L-type calcium channel blockers for use in the present invention are amlodipine, benedipine, bepridil, cinnarizine, cyclandelate, darodipine, diltiazem, etafenone, felodipine, fendiline, flunarizine, gallopamil, isradipine, lacidipine, lidoflazine, manidipine, mepirodipine, nicardipine, nifedipine,

niludipine, nilvadipine, nimodipine, nisoldipine, nitrendipine, perhexiline, piperazine, prenylamine, tiamdipine, tiapamil, verapamil, analogs thereof and pharmacologically acceptable salts thereof. The preferred blocker is diltiazem.

In some embodiments, the L-type calcium channel blocker is introduced into the intraocular chamber by instillation of a pharmacologically acceptable carrier containing said calcium channel blocker into the chamber. Preferably the L-type calcium channel blocker is introduced into the intraocular chamber by perfusing the chamber with an intraocular irrigating solution containing the blocker. The intraocular irrigation solution may contain the calcium channel blocker at a concentration of about 1 micromolar to 100 millimolar, but preferably at 0.1mM to 10.0mM. Most preferably, the intraocular irrigating solution contains diltiazem at a concentration of 0.1mM to 10mM.

The present invention also includes kits for intraocular surgery. In certain preferred embodiments, the kits comprise a package including a first container containing a first amount of an ophthalmologically acceptable carrier, the first amount being between 10ml and 1000 ml. The package also includes a second container containing an L-type calcium channel blocker in a concentrated amount. When the first amount is mixed with the concentrated amount to produce a therapeutic solution, the calcium channel blocker is present in the solution at a concentration effective for inhibiting surgical miosis or producing intraoperative mydriasis when introduced into an intraocular chamber.

In other preferred embodiments, the kit comprises a package including a first container containing a first amount of an intraocular irrigation solution, wherein the solution is incomplete with respect to one or more irrigant components. The first amount is between 100ml and 1000ml. The package also includes a housing containing an L-type calcium channel blocker in a concentrated amount and containing said one or more irrigant components in a supplement amount. When the first amount is mixed with the concentrated amount and with the supplement amount to produce a therapeutic solution, the calcium channel blocker is present in the solution at a concentration effective for inhibiting surgical miosis or producing intraoperative mydriasis when introduced into an intraocular chamber and said solution is pH and osmotically compatible with intraocular tissues. In this embodiment, the housing may be a second container containing both the L-type calcium channel blocker and the one or more irrigant components. The housing also can be a second and a third container, the second container containing the calcium channel blocker and the third container containing the one or

more irrigant comp nents.

The kits of the invention may include, in addition, instructions for preparation of the therapeutic solution and for use of the solution in connection with intraocular surgery, and in particular to inhibit miosis or produce intraoperative mydriasis during intraocular surgery.

The intraocular solutions used in the invention are water solutions containing irrigant components preferably selected from members of the group consisting of sodium ions, potassium ions, calcium ions, magnesium ions, chloride ions, acetate ions, dibasic phosphate ions, bicarbonate ions, citrate ions, dextrose and glutathione disulfide.

The present invention also sets forth a device comprising a bottle containing an intraocular solution and an L-type calcium channel blocker present in an amount effective for inhibiting miosis when perfused or instilled into an intraocular chamber of an eye during intraocular surgery. The bottle contains between 10ml and 1000ml of the intraocular solution and the calcium channel blocker, preferably at a concentration between 0.1mM and 10mM. The intraocular solution is ophthalmologically acceptable, including being pH compatible and iso-osmotic with the eye.

Other features and advantages of the invention will be apparent from the following description and from the claims.

Brief Description of the Drawings

Figure 1 is a graph illustrating the effect on pupil size of intraocular perfusion with an irrigant containing 100μM of the calcium channel blocker diltiazem.

Figure 2 is a graph illustrating the effect on pupil size of intraocular perfusion with an irrigant containing 1mM of the calcium channel blocker diltiazem.

Figure 3 is a graph illustrating the effect on post-operative intraocular pressure following intraocular perfusion with an irrigant containing 100 μM diltiazem.

Figure 4 is a graph illustrating the effect on post-operative pupil size following intraocular perfusion with an irrigant containing 100 μM diltiazem.

Figure 5 is a graph illustrating the effect on post-operative intraocular pressure following intraocular perfusion with an irrigant containing 1 mM diltiazem.

Figure 6 is a graph illustrating the effect n post-operative pupil size following intraocular perfusion with an irrigant containing 1 mM diltiazem.

Detailed Description of the Invention

This invention encompasses methods for inhibiting intraocular surgical miosis or producing intraoperative mydriasis by delivering L-type calcium channel blockers to an intraocular chamber of an eye undergoing surgery. Intraoperative miosis and surgical miosis are used interchangeably herein. Miosis means the constriction of the pupil. Miosis occurs during standard intraocular operative procedures which involve mechanical contact with ocular tissue and manipulation of ocular components. The resultant small pupil size hinders the view of the surgeon, minimizes access to the intraocular cavity and may force modification of the surgical technique. Inhibition of miosis is achieved by preventing, inhibiting or reversing the iris muscle contraction which causes a small pupil during intraocular surgery.

Mydriasis is an abnormal dilation of the pupil. Mydriasis can be useful intraoperatively by maximizing access to the intraocular cavity.

It has been discovered that L-type calcium channel blockers are capable of inhibiting intraocular surgical miosis or producing intraoperative mydriasis when applied to an intraocular chamber substantially simultaneously with surgery. While not limiting the treatment of this invention to the validity of one proposed mechanism of action, it is believed that the L-type calcium channel blockers, when introduced into an intraocular chamber, decrease the ability of the iris to contract by blocking the neuronal conduction of electrical impulses by nerves within the eye, presumably in communication with the iris.

It was known that ruthenium red can cause miosis and that this miosis was blocked by ω-conotoxin but not nifedipine. ω-conotoxin is an N-type calcium channel blocker while nifedipine is an L-type. Ruthenium red is a noxious unnatural substance which is added exogenously to the eye to induce these changes. Its mechanism of action in causing miosis is unknown, and ruthenium induced miosis very well may result from effects unrelated to nerve transmission. We believe that surgically induced miosis and ruthenium red induced miosis involve different pathways. Calcium channels are of several types, L, N, P and T. These types have varying degrees of specificity for various drugs, with different drugs acting on different channels. They are believed to be involved in different neuronal functions. The present invention involves the discovery that blockers of one such type of calcium channel, the L-type, can prevent, inhibit and even reverse the miosis induced by mechanical trauma to the eye during the course of eye surgery, even though one such blocker was shown in the prior art to be ineffective in stopping miosis induced using ruthenium red.

Intraocular chamber means a space within the eyeball which is bordered by characteristic tissues forming an identifiably separate region. The eye is comprised of three chambers: the anterior chamber, the posterior chamber and the vitreous chamber. In the method of the present invention wherein L-type calcium channel blockers are introduced into an intraocular chamber to inhibit miosis, it is believed important that the calcium channel blocker contact the nerves that activate the iris reflex. Although it is believed that the nerves activating iris contraction reside mainly in the tissue surfaces of the anterior and posterior chamber, they may travel as well to more remote intraocular surfaces such as those defining the vitreous chamber. In the present invention, the L-type calcium channel blockers preferably are brought into contact with tissues forming the anterior and posterior chamber surfaces.

The method of the invention is for treatment of surgical miosis or for producing intraoperative mydriasis in eyes of mammalian subjects (e.g., humans, nonhuman primates, dogs, cats, horses, sheep, goats, cows, pigs and rodents). Surgical procedures for which this invention is useful include, but are not limited to, the manipulation or the removal of the cataractous lens, phacoemulsification, the manipulation, insertion and/or removal of a prosthetic intraocular lens, pars plana vitrectomy, vitreal surgery, retinal surgery, extracapsular or intracapsular cataract extraction/lens aspiration and anterior segment reconstruction.

The compounds useful in practicing this invention are L-type calcium channel blockers. The term L-type "calcium channel blockers" defines a class of molecules well known to those of ordinary skill in the art. They include compounds which have been shown to prevent or delay the cardiac contracture which is caused by an accumulation of intracellular calcium. They also include compounds which have been shown to block the inward movement of extracellular Ca^{++} into a responsive cell. The term is equivalent to the terms "compound having L-type calcium channel blocking activity" or "calcium channel antagonist of the L-type".

Without limiting the invention to the specific compounds listed, the following is a list of representative L-type calcium channel blockers useful in this invention: amlodipine; benedipine; bepridil; cinnarizine; cyclandelate; darodipine; diltiazem; etafenone; felodipine; fendiline; flunarizine; gallopamil; isradipine; lacidipine; lidoflazine; manidipine; mepirodipine; nicardipine; nifedipine; niludipine; nilvadipine; nimodipine; nisoldipine; nitrendipine; perhexiline; piperazine; prenylamine; tiamdipine; tiapamil; verapamil; analogs thereof and pharmacologically acceptable salts thereof. The preferred calcium channel blocker is diltiazem

and its pharmacologically acceptable salts.

Verapamil and the like are disclosed in U.S. patents 3,261,859, 4,593,042 and 4,681,970. Nifedipine is disclosed in U.S. patent 3,485,847 and is a 1,4-dihydropyridine in which the 2 and 6 positions are substituted by methyl groups, the 4- position by 2-nitrophenyl and the 3 and 5 positions by carboxylic acid methyl ester groups. Similar compounds are disclosed in U.S. patent nos. 3,455,945, 3,325,505 and 3,441,468 to Leow and 3,470,297 and 3,511,837 to Bossert, which introduced variations in the 4-substituent. U.S. patent nos. 3,905,970 to Bossert, et al. and 3,985,758 to Marakami et al. introduced certain mono-or dialkylamino-alkylene and nitrogen-containing heterocyclic alkylene groups into one or both of the 3,5 ester groups. U.S. Patent No. 4,307,103 and 4,393,070 to Sato disclose 1,4-dihydropyridines in which the 2 position is not substituted by alkyl, but instead is substituted with cyano, formyl or certain other substituents and the ester group in the 3 position may contain various substituted alkyl groups including substituted alkylaminoalkyl, heterocyclic aminoalkyl and aroylaminoalkyl, including phthalminidoethyl. U.S. Patent No. 4,448,964 to Muto et al. discloses compounds in which the 3-position ester group contains certain substituted piperidinyl alkylene groups.

Other pyridine compounds having calcium channel blocking activities are disclosed in U.S. Patents 4,652,573, 4,755,512, 4,791,117, 4,794,187, 4,814,455, 4,829,076, 4,871,745, 4,895,846 and 4,912,223.

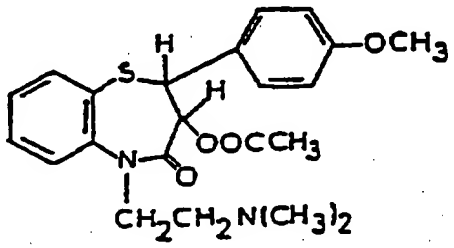
Diltiazem and analogs are disclosed in U.S. patents 3,562,257 and 4,552,695.

Analogs of the foregoing compounds that function as L-type calcium channel blockers also are specifically intended to be embraced by this invention. An analog is a molecule that is structurally similar to the parent molecule and is capable of achieving the same or substantially the same function or activity in terms of miosis or mydriasis. The ability of such analogs to prevent, inhibit or reverse surgical miosis or produce intraoperative mydriasis according to the invention can be tested easily using no more than routine experimentation.

Pharmaceutically acceptable salts of L-type calcium channel blockers include the conventional non-toxic salts formed from non-toxic inorganic or organic acids. For example, such conventional non-toxic salts include those derived from inorganic acids such as hydrochloric, hydrobromic, sulfuric, sulfamic, phosphoric, nitric and the like; and the salts prepared from organic acids such as acetic, proprionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, pamoic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic,

salicylic, sulfanilic, 2-acetoxybenz ic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isethionic, formic, malonic, naphthalene-2-sulf nic, benzenesulfonic and the like.

The structure of the preferred calcium channel blocker utilized in the method and compositions of this invention is as follows:



Diltiazem

The chemical name of diltiazem (d-diltiazem) is (2S,3S)-3-acetoxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-(methoxyphenyl)-1,5-benzothi azepin-4(5H)-one hydrochloride. The chemical name of the l-enantiomer of diltiazem is (2R,3R)-3-acetoxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4(methoxyphenyl)-1,5-benzo-thiazepin-4(5H)-one hydrochloride.

Analogs of diltiazem include: 1,3,4,5-tetrahydro-3-(methoxycarbonyl)-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one; 1,3,4,5-tetrahydro-3-hydroxy-3-(methoxycarbonyl)-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one;1,3,4,5-tetrahydro-hydroxy-4-(4-methoxycarbonyl)-2H-1-benzazepin-2-one; trans-1,3,4,5-tetrahydro-3-hydroxy-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one; cis-3-hydroxy-1-[2-(dimethylamino)ethyl]-1,3,4,5-tetrahydro-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one, monohydrochloride;cis-3-(acetyloxy)-1-[2-(dimethylamino)ethyl]-1,3,4,5-tetrahydro-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one, monohydrochloride; 1,3,4,5-tetrahydro-3-methyl-3-(methoxycarbonyl)-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one; cis-1,3,4,5-tetrahydro-3-methyl-3-(methoxycarbonyl)-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one;cis-3-methyl-1-[2-(dimethylamino)ethyl]-1,3,4,5-tetrahydro-4-(4-methoxyphenyl)-2H-1-benzazepin-2-one, monohydrochloride;

1,3,4,5-tetrahydro-2-(4-methoxyphenyl)-3-methyl-4-oxo-1,5-benzothiazepine-3-carboxylic acid, methyl ester; 1,3,4,5-tetrahydro-2-(4-methoxyphenyl)-3-methyl-4-oxo-1,5-benzothiazepine-3-carboxylic acid;cis-2,3-dihydro-2-(4-methoxyphenyl)-3-methyl-1,5-benzothiazepin-4(5H)-one;cis-5-[2-dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-3-methyl-1,5-benzothiazepin-4(5H)-one hydrochloride; 3-hydroxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride; 3-hydroxy-2,3-dihydro-5-[2-(methylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride; 3-hydroxy-2,3-dihydro-5-[2-(dimethylamino)ethyl]-2-(4-hydroxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride; 3-hydroxy-2,3-dihydro-5-[2-(methylamino)ethyl]-2-(4-hydroxyphenyl)-1,5-benzothiazepine-4(5H)-one hydrochloride; 3-acetoxy-8-chloro-2,3-dihydro-5-[2-dimethylamino)ethyl]-2-(4-methoxyphenyl)-1,5-benzothiazepine-4(5)-one maleate (1:1).

The L-type calcium channel blockers are introduced into an intraocular chamber in ophthalmologically acceptable amounts and in ophthalmologically acceptable solutions. Such amounts and solutions are those that cause no medically unacceptable side-effects when administered to an intraocular chamber of the eye according to the methods described herein. Preferred ophthalmologically acceptable solutions are sterile solutions which are approximately iso-osmotic with respect to the fluid in intraocular chambers. Such solutions are non-irritating to the eye and maintain the osmotic stability of the tissues defining the chamber. The osmolality preferably is between about 250 and about 350 mOsm and most preferably about 280-320 mOsm. The solutions also are pH compatible with the environment of the selected intraocular chamber. The pH of the solution preferably is between about 6.5 and about 8.0 and more preferably between about 7.2-7.8. Most preferably the pH is 7.4. The solutions optionally contain particular buffering agents and other factors to support metabolism of the eye tissue. For example, the solution may contain bicarbonate at a concentration of between about 10 and 50 mM/l. The solution also may contain, for example, dextrose (D-glucose) and glutathione. The buffer preferably is a phosphate buffer whereby the final phosphate concentration is between about 1 and 5 mM/l. Other additives include sodium and potassium salts such as sodium and potassium chlorides, sulfates, acetates, citrates, lactates, and

gluconates. Calcium and magnesium chlorides also may be added.

The L-type calcium channel blocker is introduced into the intraocular chamber in an ophthalmologically acceptable carrier solution containing the calcium channel blocker at an effective concentration for inhibiting surgical miosis or producing intraoperative mydriasis. Introduced into the chamber means instilling it in the chamber or perfusing it in the chamber. The solution containing the calcium channel blocker can be instilled in an intraocular chamber using a syringe at a time prior to the start of surgery or very early in the procedure. This instillation may be a single application of a small amount of carrier solution containing the calcium channel blocker. When instilled in an intraocular chamber prior to surgery, the L-type calcium channel blocker is intended to exert its effect for the total time anticipated for the surgical procedure. The solution thus contains amounts of an L-type calcium channel blocker sufficient to assure persistent inhibition of miosis or continuous mydriasis during the surgery. The solution also may be instilled into the chamber as a "wash", once or several times during the surgery.

The eye also may be perfused during the course of the surgery with an ophthalmologically acceptable irrigation solution containing the L-type calcium channel blocker. Perfusion is accomplished by means of a perfusing needle, cannula or probe which delivers in a sterile manner perfusing solution from a container. The cannula (as generally used in intraocular surgery) is capable of both providing the irrigation solution to the eye and also aspirating fluid thereby maintaining a clear field of operation for the surgeon.

The duration of action of the L-type calcium channel blocker can influence the time at which the blocker is introduced into a chamber of the eye undergoing surgery. It is intended that the L-type calcium channel blocker be applied substantially simultaneously with the surgical procedure. "Substantially simultaneously" means that the blocker is introduced such that its effect coincides with the time during which the surgical procedure is being performed.

The duration of action of the blocker will affect the choice of the blocker and the method by which the blocker is introduced into the intraocular chamber. Thus, an L-type calcium channel blocker which is capable of exhibiting an effect for about a half hour or more (i.e., the entire time which may be needed to perform the eye surgery) may be introduced into an intraocular chamber in a single dose prior to or at the beginning of surgery. Those L-type calcium channel blockers that have a somewhat shorter duration of action may need re-instillation, such as by a wash. In a preferred embodiment, however, the blockers have a

short duration of action and are introduced into the intraocular chamber by constant perfusion of an intraocular chamber during the course of surgery.

The L-type calcium channel blockers of the invention are applied in effective amounts. An effective amount is that amount which prevents, inhibits or reverses miosis or produces mydriasis to a medically useful extent during intraocular surgery, (e.g. the operative field is improved for the surgeon or it is easier for the surgeon to manipulate surgical instruments and intraocular tissues). An effective amount is one controlled by a number of factors, including: the subject; the inherent anti-miotic or mydriatic activity of the L-type calcium channel blocker; the amount of the L-type calcium channel blocker used; its duration of action; and the method by which it is introduced into the intraocular chamber, i.e., whether by a single application or by continuous perfusion. A perfusion solution having between 1 micromolar to 100mM of L-type calcium channel blocker is believed to deliver an effective amount during intraocular surgery. Preferably perfusion solutions contain between 0.1mM to 10mM blocker. It is important that the L-type calcium channel blockers do not produce, at effective concentrations, long-term deleterious changes in the eye nor cause inflammation, discomfort or irritation. The determination of an effective dose for any selected compound is well within the level of ordinary skill in the art.

The present invention also includes kits providing an L-type calcium channel blocker and a carrier for introducing the blocker into an intraocular chamber during surgery. A carrier is an ophthalmologically acceptable solution in which the blocker is dissolved prior to application to an intraocular chamber. It is important that any solution applied to an intraocular cavity be free of any factors that would injure intraocular tissue. Thus it preferably is sterile, approximately iso-osmotic, at the correct pH and contains factors to support metabolism in the tissue, as described above.

The long-term effects of storage on the stability of L-type calcium channel blockers that contain, for example, an ester function within the structure are believed to be problematic, particularly when the blockers are in aqueous solutions at neutral or alkaline pH. Accordingly, it is an aspect of the invention that kits be provided for preparation of therapeutic solutions containing an L-type calcium channel blocker within hours of surgery. In these kits, the blockers can be in a stable powdered form or in a stable concentrated form such as concentrated in an acidic solution. The powdered or concentrated L-type calcium channel

blocker then can be dissolved r diluted in the ophthalmologically acceptable carrier solution immediately in advance of surgery.

The kits comprise a package, such as a box, blister pack or similar packing vehicle used conventionally to hold containers of liquid. The package may be coated with an impervious cover to assist in protecting the sterility of the contents during transport and storage. In the package are a container containing an amount of a carrier solution (or components thereof) and one or more containers containing an L-type calcium channel blocker and any components missing from the carrier solution. The containers preferably are glass bottles, but may be formed of any inert material such as a rigid or flexible plastic in the form of bottles or bags that allow transport and storage of liquid without loss of fluid or contamination of the contents.

In certain preferred embodiments, the containers may be chambers in a single housing. In these embodiments the container may comprise in addition a structure to permit communication of the contents of the chambers without opening the container. In one such embodiment, the blocker and the ophthalmologically acceptable carrier solution can be supplied in separate chambers of a two-chamber vial. Communication between the chambers can be provided by a frangible membrane. In use, the membrane is pierced or ruptured, with the carrier solution flowing into the chamber containing the blocker (or vise versa). The blocker, in powdered form or in a concentrated form, then is dissolved or diluted into the solution. In another embodiment, the upper and lower chambers are constructed and arranged within a syringe. Movement of the plunger causes the contents of the two chambers to mix.

In one aspect of the invention, the kit may be used to provide a carrier solution for instilling a single amount or wash amounts of an L-type calcium channel blocker into an intraocular chamber. In these embodiments, the containers for the carrier solution preferably includes about 10ml to about 50ml of carrier solution. The container may be a bottle or vial with piercable septum. The blocker may be supplied for example in a stable, concentrated solution (also in a bottle or vial with a piercable septum). In use, the septum of the bottle or vial containing the calcium channel blocker is pierced by the needle of a syringe and transferred to the bottle or vial containing the carrier solution. An ophthalmologically acceptable solution of predetermined blocker concentration effective for inhibiting surgical miosis or producing intraoperative mydriasis when introduced into an intraocular chamber is thereby formed. The solution then may be removed by syringe from the vial and instilled into the intraocular chamber.

In another aspect of the invention, the kit may be used to provide a carrier for perfusing an L-type calcium channel blocker into an intraocular chamber during eye surgery. In these embodiments, the first container contains an intraocular irrigation solution (or components thereof) in an amount from about 100ml to 1000ml, preferably about 500ml. Most preferably the first container is a bottle having a rubber septum which can be punctured by a needle attached to a tube for delivering the contents of the container to a perfusing needle and hence to the eye. Another container(s) includes a predetermined amount of an L-type calcium channel blocker, and, in certain embodiments, components of the intraocular irrigation solution. The contents of the containers are mixed in a manner that maintains sterility to form an ophthalmologically acceptable solution containing the blocker at a concentration effective for inhibiting surgical miosis when perfused into an intraocular chamber during surgery.

As mentioned above, the solution contained in the first container may be only components of an intraocular irrigation solution. In other words, the intraocular irrigation solution may be "incomplete". One or more components of the intraocular irrigation solution may be provided in a second container with the blocker or separately in a different container. Such arrangements can serve dual purposes. Firstly, certain components of the intraocular irrigation solution, such as organic components, may be more stable at pHs other than physiological intraocular pH. Thus, as with the blocker, the separation of such one or more components from the irrigation solution (and the calcium blocker solution) until the time of mixing just prior to surgery permits long term storage. Secondly, by providing important components in separate containers, a package may be constructed and arranged whereby an ophthalmologically acceptable irrigation solution is created only when all of the various contents of the different containers are mixed together. In this manner, the surgeon or medical staff supporting the surgeon will be inclined to use the materials as directed as opposed to substituting other materials which may not be as clinically desirable.

Thus, in certain preferred embodiments of the kit of the invention, the package includes a first container containing between 100ml and 1000ml of an intraocular solution, wherein the solution is incomplete with respect to one or more solution components. The package also includes a second container containing an L-type calcium channel blocker and a third container containing said one or more solution components. In these embodiments, the contents of the first, second, and third containers are mixed together to form a solution which is pH and osmotically compatible with the intraocular environment of an eye and in which the blocker is

present at a concentration effective for inhibiting surgical miosis when introduced into an intraocular chamber.

Examples of suitable intraocular irrigation solutions are RINGERS solution, balanced salt solution and glutathione-bicarbonate-RINGERS solution. The preferred compositions and methods of preparation of suitable irrigation solutions have been disclosed in U.S. patent numbers 4,550,022 and 4,443,432 to Garabedian which are hereby incorporated by reference.

The kits may include instructions for preparation of a carrier or irrigation solution. The instructions may detail the use of the L-type calcium channel blocker or solution in an intraocular chamber in connection with inhibiting surgical miosis. They also may include useful additional implements for mixing the contents of the containers in the kits or for delivery of the final therapeutic solution to an intraocular chamber.

The present invention also sets forth a device comprising a bottle, such as a wash bottle or an irrigation bottle, containing an intraocular irrigation solution including an L-type calcium channel blocker present in an amount effective for inhibiting miosis or producing mydriasis when instilled into an intraocular chamber of an eye during intraocular surgery. The bottle preferably is formed from glass, but may also be rigid or flexible plastic and may be a bag. The device is useful for instilling or perfusing a solution in an intraocular chamber during surgery. A bottle suitable for wash or injection preferably contains 10ml to 50ml of the ocular irrigating solution and preferably contains the blocker at a concentration between about 0.1mM and 10mM. A bottle suitable for use in perfusion of an intraocular cavity contains at least 100 ml of the intraocular irrigation solution and preferably contains the blocker at a concentration between about 1 micromolar to 10mM.

Example 1

Lens removal surgery in dogs. All dogs underwent a presurgical work up including a complete physical exam, a complete ocular exam, an intraocular pressure measurement and a pupil measurement using a hand-held caliper (measured to the nearest millimeter).

The dogs were treated with the following anti-inflammatory regimen: on the morning before surgery, the dogs were given .5 mg/lb prednisone PO, one drop of .1% dexamethazone with neomycin and polymixin B (AK-Trol™) and .25 mg/lb flunixin meglumine IV. Beginning one hour before surgery, and every 15 minutes thereafter up until surgery, one drop of AK-Trol was applied topically. Post-operatively, QID one drop .1% AK-Trol Sx day. This was repeated at 1, 2 and 3 days post-operatively.

Surgical removal of the lens was performed using the techniques of phacoemulsification in mongrel dogs of either sex. General anesthesia was induced by intravenous Pentothal (25mg/ml, at a dose of 8mg/lb) and maintained by inhalation with isoflurane. The surgical site was prepared, draped and washed with BETADINE. Surgery was performed on one eye, then the other. Lactated RINGERS solution was administered IV during the procedure, 4ml/lb/hr.

The surgical procedure was performed by observation through a surgical microscope. The anterior chamber was entered at the limbus and an anterior capsulotomy was performed. Using the phacoemulsification probe and irrigation/aspiration, the lens content material was broken up and removed from the eye. The irrigation solution was a balanced salt solution containing either the drug substance or a vehicle control. Over 15-20 minutes of surgical time, 100-400 mls of irrigation solution were delivered. Pupil size was monitored under the surgical microscope with the hand-held caliper.

At the end of surgery, the eye was closed with sutures and surgery on the other eye was performed similarly. When the second procedure was completed, the animal was allowed to recover. Post-operative inflammation was measured with a KOWA™ Flarmeter for 72 hours after surgery.

Following the foregoing protocol, three dogs were treated in one eye with a vehicle and in the other eye with a perfusion solution containing 100 micromolar diltiazem. The results are shown in Fig. 1 and TABLE 1, which demonstrates that pupil size was increased only slightly between the 10 and 20 minute interval after the initiation of the surgical procedure. (The perfusion solution was applied beginning at seven minutes.) The results were as follows:

TABLE 1

Pupil Diameter (mm)

Time (minutes)	Diltiazem (100μM)	Placebo
Pre-Operative	8.67	8.67
Post-Anesthesia	3.00	3.17
0	3.67	3.75
5	3.13	4.56
10	6.20	3.88
15	5.75	5.30
20	5.93	5.40
25		
Post-Closure	4.42	4.42

Example 2

The protocol of Example 1 was followed, except that three dogs were treated in one eye with a vehicle and in the other eye with a perfusion solution containing 1 millimolar diltiazem. As demonstrated in Fig. 2 and TABLE 2, the eye receiving the diltiazem had a pupil size substantially greater than the eye receiving the placebo. The results were as follows:

TABLE 2

Pupil Diameter (mm)

Time (minutes)	Diltiazem (1mM)	Placebo
Pre-Operative	9.00	9.00
Post-Anesthesia	3.50	4.00
0	4.50	3.63
5	9.20	4.30
10	8.33	4.75
15	10.75	3.00
20	9.63	4.17
25	9.33	3.83
Post-Closure		

Example 3

The effect 100μM of diltiazem on post-operative intraocular pressure (IOP) was measured. No statistical differences were apparent between the eyes treated with 100μM diltiazem and the eyes treated with the placebo. The results are shown in Fig. 3.

Example 4

The effect of 100μM diltiazem on post-operative pupil size also was measured. No statistical differences were observed in eyes treated with 100μM diltiazem versus eyes treated with placebo through 72 hours post-operatively. The results are shown in Fig. 4.

Example 5

The effect of 1mM diltiazem on post-operative intraocular pressure (IOP) was measured. No statistical differences were apparent between the eyes treated with 1mM diltiazem and the eyes treated with the placebo. The results are shown in Fig. 5.

Example 6

The effect of 1mM diltiazem on post-operative pupil size also was measured. No statistical differences were observed in eyes treated with 1mM diltiazem versus eyes treated with placebo through 72 hours post-operatively. The results are shown in Fig. 6.

While the invention has been described in terms of preferred embodiments, those of ordinary skill in the art will recognize that modifications and equivalents may be made without departing from the scope of the present invention which is limited only by the following claims:

CLAIMS

1. A method for inhibiting intraoperative miosis or producing intraoperative mydriasis comprising introducing into an intraocular chamber of a subject, substantially simultaneously with performing intraocular surgery on said subject, an amount of an L-type calcium channel blocker effective for inhibiting surgical miosis or producing intraoperative mydriasis.

2. The method of claim 1 wherein said L-type calcium channel blocker is selected from the group consisting of amlodipine, benedipine, bepridil, cinnarizine, cyclandelate, darodipine, diltiazem, etafenone, felodipine, fendiline, flunarizine, gallopamil, isradipine, lacidipine, lidoflazine, manidipine, mepirodipine, nicardipine, nifedipine, niludipine, nilvadipine, nimodipine, nisoldipine, nitrendipine, perhexiline, piperazine, prenylamine, tiamdipine, tiapamil, verapamil, analogs thereof and pharmacologically acceptable salts thereof.

3. The method of claim 1 wherein said L-type calcium channel blocker is introduced into said intraocular chamber by perfusing said chamber during intraocular surgery with an intraocular irrigating solution containing said blocker.

4. The method of claim 2 wherein said L-type calcium channel blocker is introduced into said intraocular chamber by perfusing said chamber during intraocular surgery with an intraocular irrigating solution containing said L-type calcium channel blocker.

5. The method of claim 1 wherein said L-type calcium channel blocker is introduced into said intraocular chamber by injection of a pharmacologically acceptable carrier containing said L-type calcium channel blocker into said intraocular chamber.

6. The method of claim 2 wherein said L-type calcium channel blocker is introduced into said intraocular chamber by injection of a pharmacologically acceptable carrier containing said L-type calcium channel blocker into said intraocular chamber.

7. The method of claim 3 or 4 wherein said L-type calcium channel blocker is diltiazem, an analog of diltiazem, or pharmacologically acceptable salts thereof.

8. The method of claim 3 or 4 wherein said L-type calcium channel blocker is at a concentration of 1 micromolar to 100mM in said intraocular irrigating solution.

9. The method of claim 5 or 6 wherein said L-type calcium channel blocker is at a concentration of 0.1mM to 10mM in said carrier.

10. A kit for intraocular surgery comprising a package including:

a. a first container containing a first amount of an ophthalmologically acceptable carrier, the first amount being between 10ml and 1000ml; and

b. a second container containing an L-type calcium channel blocker in a concentrated amount,

wherein when the first amount is mixed with the concentrated amount to produce an ophthalmologically acceptable solution, the L-type calcium channel blocker is present in said ophthalmologically acceptable solution at a concentration effective for inhibiting intraoperative miosis or producing intraoperative mydriasis when perfused in an intraocular chamber.

11. A kit for providing an irrigant for intraocular surgery comprising a package including:

a. a first container containing an irrigation amount of an intraocular irrigation solution, wherein the solution is incomplete with respect to one or more irrigant components, the irrigation amount being between 100 ml and 1000 ml; and

b. a housing containing an L-type calcium channel blocker in a concentrated amount and containing said one or more irrigant components in a supplement amount;

wherein, when the irrigation amount is mixed with the concentrated amount and with the supplement amount to produce an ophthalmologically acceptable solution, the L-type calcium channel blocker is present in said ophthalmologically

acceptable solution at a concentration effective for inhibiting intraoperative miosis or producing intraoperative mydriasis when perfused into an intraocular chamber during intraocular surgery, and said ophthalmologically acceptable solution is pH and osmotically compatible with intraocular tissues.

12. A kit for producing an irrigant for intraocular surgery as claimed in claim 11, wherein said housing is two containers, a second container containing said one or more irrigant components in the supplement amount; and a third container containing the L-type calcium channel blocker in the concentrated amount.

13. The kit of claims 10, 11 and 12 further comprising instructions for preparation of said ophthalmologically acceptable solution and for use of said ophthalmologically acceptable solution as an irrigant during intraocular surgery.

14. The kit of claims 10, 11 and 12 wherein said ophthalmologically acceptable solution is a water solution containing components selected from members of the group consisting of sodium ion, potassium ion, calcium ion, magnesium ion, chloride ion, acetate ion, dibasic phosphate ion, bicarbonate ion, citrate ion, dextrose and glutathione disulfide, said solution being adjusted to a pH of between 6.5 and 8.

15. The kit of claims 10, 11 and 12 wherein said L-type calcium channel blocker is selected from the group consisting of amlodipine, benedipine, bepridil, cinnarizine, cyclandelate, darodipine, diltiazem, etafenone, felodipine, fendiline, flunarizine, gallopamil, isradipine, lacidipine, lidoflazine, manidipine, mepirodipine, nicardipine, nifedipine, niludipine, nilvadipine, nimodipine, nisoldipine, nitrendipine, perhexiline, piperazine, prenylamine, tiamdipine, tiapamil, verapamil, analogs thereof and pharmacologically acceptable salts thereof.

16. The kit of claim 15 wherein said L-type calcium channel blocker is diltiazem, an analog of diltiazem, or pharmacologically acceptable salts thereof.

17. A device comprising a bottle containing an intraocular irrigating solution and an L-type calcium channel blocker present in an amount effective for inhibiting miosis or producing intraoperative mydriasis when perfused into an intraocular chamber of an eye during intraocular surgery.

18. The device claimed in claim 17 wherein said bottle contains at least 100ml of said intraocular irrigating solution.

19. The device of claim 18 wherein said bottle contains an L-type calcium channel blocker at a concentration between 0.1mM and 10mM.

20. The device of claim 19 wherein said L-type calcium channel blocker is diltiazem, an analog of diltiazem, or pharmacologically acceptable salts thereof.

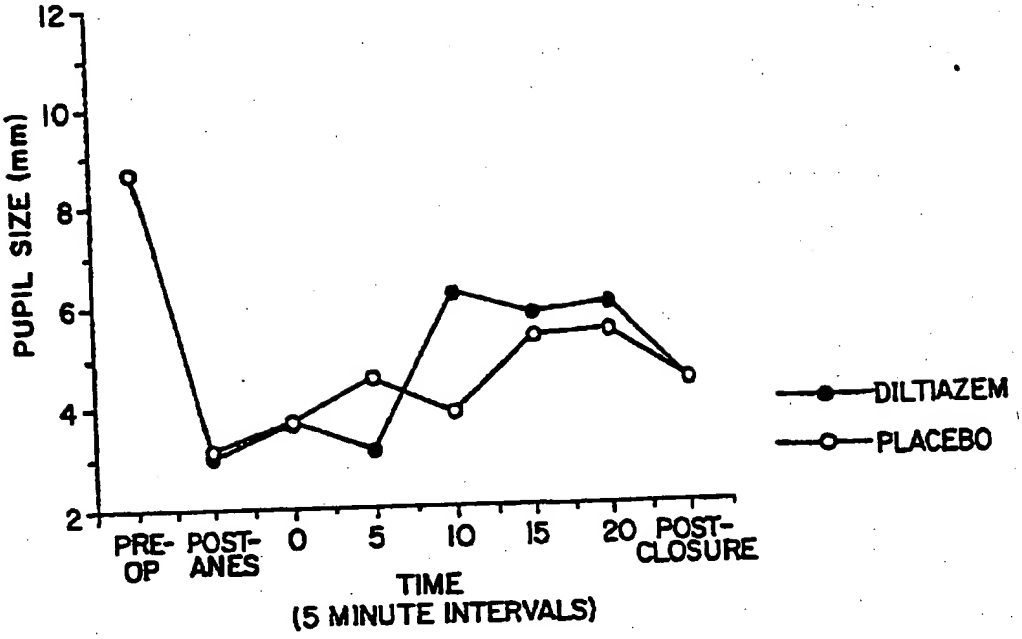

12
10
8
6
4
2
PUPIL SIZE (mm)
PRE-OP
POST-ANES
0
5
10
15
20
POST-CLOSURE
TIME
(5 MINUTE INTERVALS)
DILTIAZEM
PLACEBO

Fig. 1

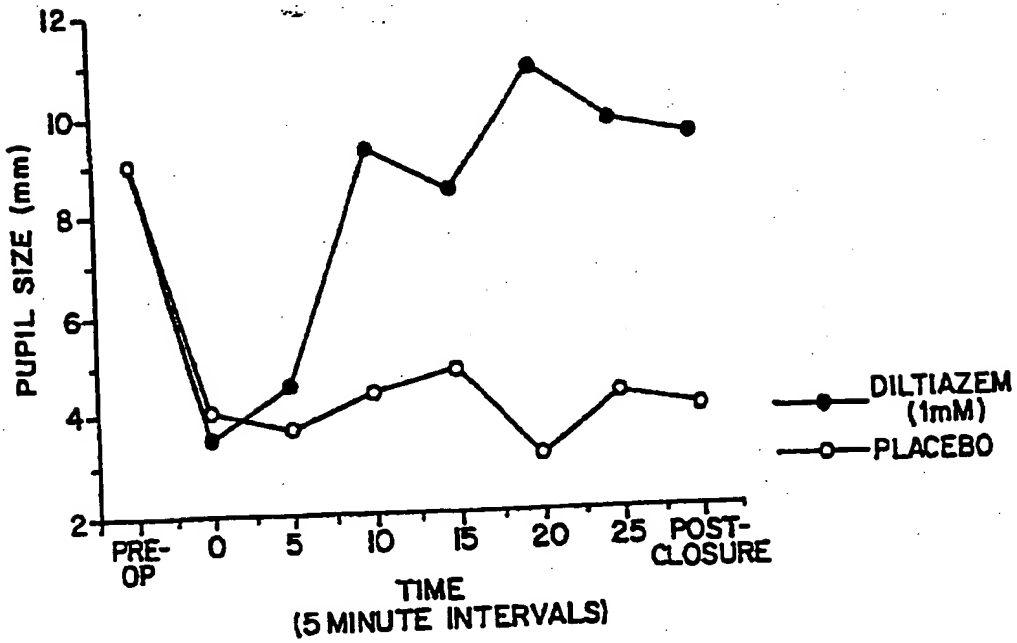

12
10
8
6
4
2
PUPIL SIZE (mm)
PRE-OP
0
5
10
15
20
25
POST-CLOSURE
TIME
(5 MINUTE INTERVALS)
DILTIAZEM (1mM)
PLACEBO

Fig. 2

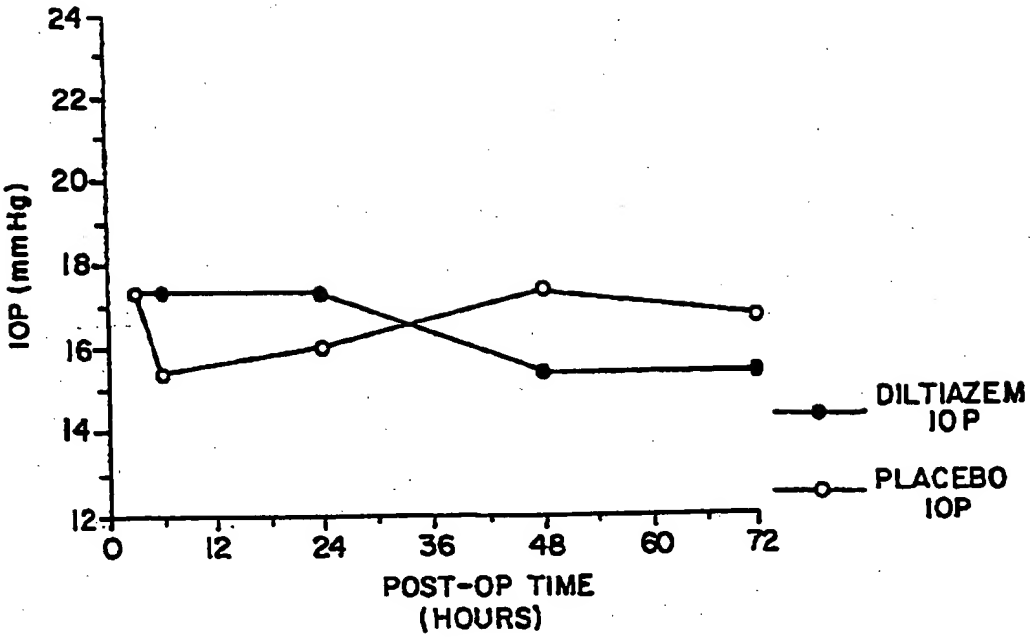

IOP (mmHg)
24
22
20
18
16
14
12
0
12
24
36
48
60
72
POST-OP TIME
(HOURS)
DILTIAZEM
IOP
PLACEBO
IOP

Fig. 3

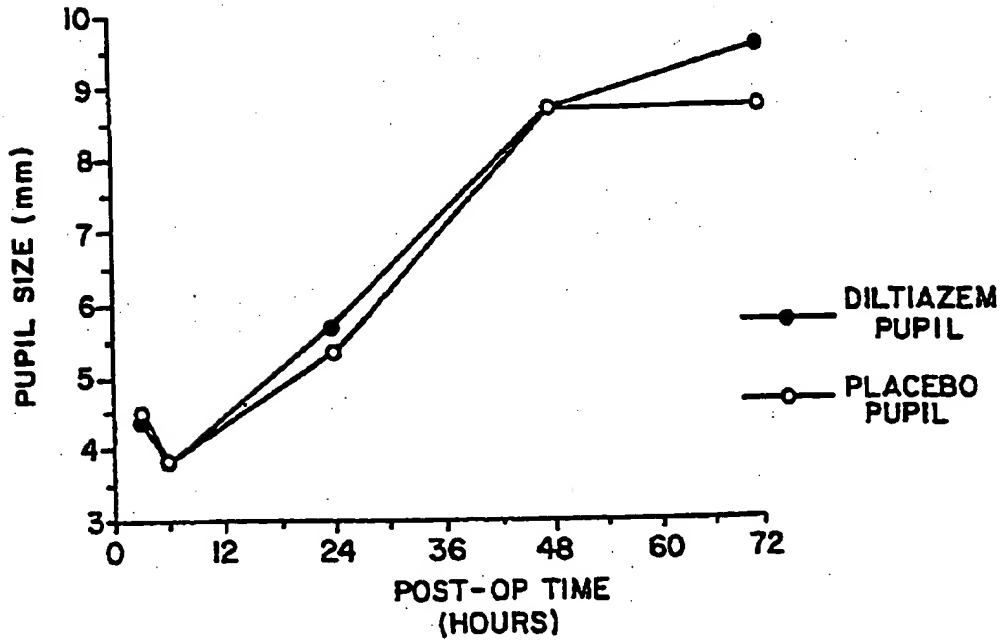

PUPIL SIZE (mm)
10
9
8
7
6
5
4
3
0
12
24
36
48
60
72
POST-OP TIME
(HOURS)
DILTIAZEM
PUPIL
PLACEBO
PUPIL

Fig. 4

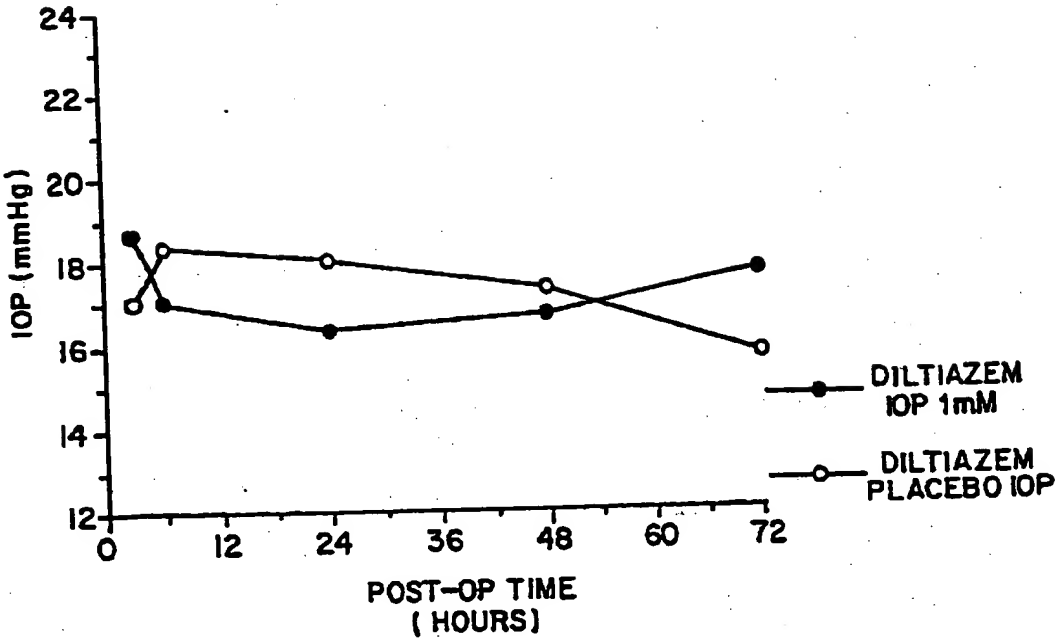

24
22
20
18
16
14
12
IOP (mmHg)
0
12
24
36
48
60
72
POST-OP TIME
(HOURS)
DILTIAZEM
IOP 1mM
DILTIAZEM
PLACEBO IOP

Fig. 5

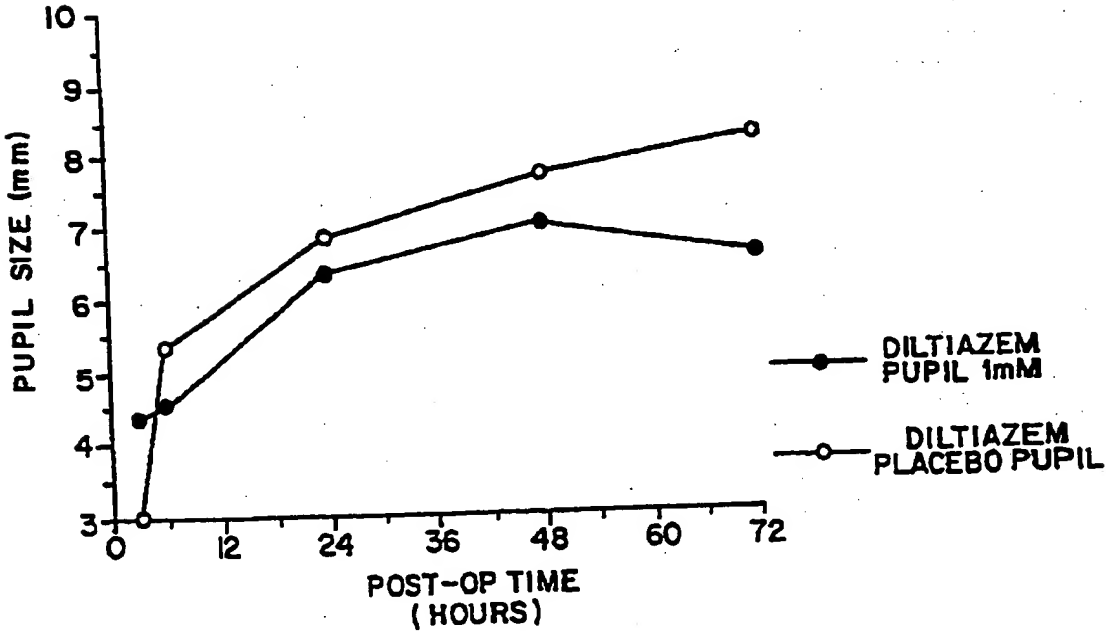

10
9
8
7
6
5
4
3
PUPIL SIZE (mm)
0
12
24
36
48
60
72
POST-OP TIME
(HOURS)
DILTIAZEM
PUPIL 1mM
DILTIAZEM
PLACEBO PUPIL

Fig. 6

INTERNATIONAL SEARCH REPORT

Internatbo \pplicatiao No
PCT/US 95/07526

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/135 A61K31/275 A61K31/44 A61K31/445 A61K31/495 A61K31/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,92 04008 (UNIVERSIDAD DE ALICANTE) 19 March 1992 see page 15 - page 16; example 6	17-20
Y	see page 17, line 14 - line 15	1-16
X	PHARMACOLOGY, BIOCHEMISTRY & BEHAVIOR, vol. 24, 1986 pages 329-331, BELESLIN D.B. ET AL 'Verapamil-Induced Behavioral, Autonomic and Motor Effects in Cats' see page 329, right column, line 9 - line 10	17-20
Y	see abstract	1-16
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 1995	19.10.95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Seegert, K

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US 95/07526

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	'Rote Liste' 1993 , EDITIO CANTOR , AULENDORF/WURTT. see example 26104 see example 26119 see example 26124	10-20
X	FR,A,2 593 395 (CORBIERE) 31 July 1987 see page 4 - page 5; examples I-V	10-20
E	WO,A,95 15958 (ALCON LAB.) 15 June 1995 see page 43, line 14 - page 44, line 6 see page 69, line 1 - line 16	10-14, 17-19
A	EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 209, no. 3, 1991 pages 175-183, ANDERSSON S.E. ET AL 'Ruthenium Red and Capsicain Induce a Neurogenic Inflammatory Response in the Rabbit Eye: Effects of Conotoxin GVIA and Tetrodotoxin' cited in the application see page 181, right column, line 10 - line 13	1-20

1

INTERNATIONAL SEARCH REPORT

Internatio Application No
PCT/US 95/07526

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9204008	19-03-92	AU-A-	8397191	30-03-92
		CA-A-	2073003	08-03-92
		EP-A-	0502143	09-09-92
FR-A-2593395	31-07-87	NONE		
WO-A-9515958	15-06-95	AU-B-	1336895	27-06-95

AST 3

United States Patent [19]

Alker et al.

[11] **Patent Number: 4,590,195**

[45] **Date of Patent: May 20, 1986**

[54] **4-ARYL-3,5-BIS(ALKOXYCARBONYL)-6-METHYL-2-AMINOALKYLOXYMETHYL-1,4-DIHYDROPYRIDINE ANTIHYPERTENSIVE AGENTS**

[75] Inventors: **David Alker, Eastry, Nr. Deal; Peter E. Cross, Canterbury; Simon F. Campbell, Deal, all of England**

[73] Assignee: **Pfizer Inc., New York, N.Y.**

[21] Appl. No.: **586,514**

[22] Filed: **Mar. 5, 1984**

[30] **Foreign Application Priority Data**

Mar. 10, 1983 [GB] United Kingdom 8306666

[51] Int. Cl.[4] C07D 401/12; C07D 211/82; C07D 213/55; A61K 31/495; A61K 31/44

[52] U.S. Cl. 514/252; 544/364; 544/124; 544/310; 544/336; 546/255; 546/153; 546/193; 546/256; 546/261; 546/262; 546/270; 546/271; 546/274; 546/278; 546/280; 546/282; 546/283; 546/340; 546/341; 514/256; 514/314; 514/318; 514/332; 514/333; 514/335; 514/336; 514/338; 514/339; 514/341; 514/342; 514/343; 514/356

[58] Field of Search 546/256, 261, 262, 270, 546/271, 274, 278, 280, 282, 283, 340, 341; 424/263; 544/364; 514/252

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,974,278	8/1976	Bossert et al.	546/262
4,380,547	4/1983	Materne	546/270
4,430,333	2/1984	Campbell et al.	424/266

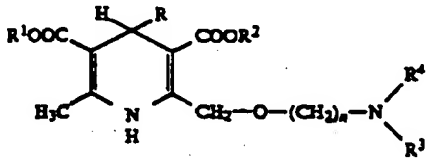
FOREIGN PATENT DOCUMENTS

0031801	7/1981	European Pat. Off.
0060674	9/1982	European Pat. Off.
0089167	9/1983	European Pat. Off.
55-47656	4/1980	Japan .
1585978	3/1981	United Kingdom .

Primary Examiner—George F. Lesmes
Assistant Examiner—S. A. Gibson
Attorney, Agent, or Firm—Charles J. Knuth; Albert E. Frost; James M. McManus

[57] **ABSTRACT**

1,4-Dihydropyridine derivatives of the formula:



wherein R is aryl or heteroaryl; R^1 and R^2 are each C_1–C_4 alkyl or 2-methoxyethyl; n is 2, 3 or 4; R^3 is H, C_1–C_4 alkyl, $CH_2CO_2(C_1$–C_4 alkyl) or CH_2CN; and R^4 is a group of the formula COR^5, CSR^5, $C(=NR^6)R^7$ or SO_2R^5, wherein R^5 is C_1–C_4 alkyl, NH_2, $NH(C_1$–C_4 alkyl), $NH(C_3$–C_6 cycloalkyl), $N(C_1$–C_4 alkyl)$_2$, $NHCH_2CO_2(C_1$–C_4 alkyl), $NHCH_2CONH_2$, $NHCH_2CO_2H$, $NH(CH_2)_2NH_2$, $NHNH_2$, $NHNRCO_2(C_1$–C_4 alkyl), NH-aryl, NHCO-aryl or a heterocyclic, NH-heterocyclic or NHCO-heterocyclic group, or when R^4 is $C(=O)R^5$, R^5 may be H or CF_3; R^6 is H, CN, $CO_2(C_1$–C_4 alkyl), $CO(C_1$–C_4 alkyl), $SO_2(C_1$–C_4 alkyl), SO_2-aryl, SO_2NH_2, $SO_2N(C_1$–C_4 alkyl)$_2$, NO_2 or aryl; and R^7 is NH_2, $NH(C_1$–C_4 alkyl), $NHCO(C_1$–C_4 alkyl), $NH(CH_2)_mN(C_1$–C_4 alkyl)$_2$ wherein m is 2 to 4 or a NH-heterocyclic group; and their pharmaceutically acceptable acid addition salts, and pharmaceutical preparation containing such compounds, have utility as anti-ischaemic and antihypertensive agents.

6 Claims, No Drawings

4-ARYL-3,5-BIS(ALKOXYCARBONYL)-6-METHYL-2-AMINOALKYLOXYMETHYL-1,4-DIHYDROPYRIDINE ANTIHYPERTENSIVE AGENTS

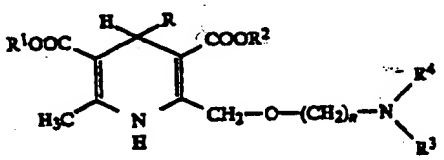
BACKGROUND OF THE INVENTION

This invention relates to certain dihydropyridines, specifically to certain 1,4-dihydropyridines having a nitrogen containing group in a side chain attached to the 2-position, which have utility as anti-ischaemic and antihypertensive agents, and to pharmaceutical preparations containing such compounds.

The compounds of the invention reduce the movement of calcium into the cell and they are thus able to delay or prevent the cardiac contracture which is believed to be caused by an accumulation of intracellular calcium under ischaemic conditions. Excessive calcium influx during ischaemia can have a number of additional adverse effects which would further compromise the ischaemic myocardium. These include less efficient use of oxygen for ATP production, activation of mitochondrial fatty acid oxidation and possibly, promotion of cell necrosis. Thus the compounds are useful in the treatment or prevention of a variety of cardiac conditions, such as angina pectoris, cardiac arrythmias, heart attacks and cardiac hypertrophy. The compounds also have vasodilator activity since they can inhibit calcium influx in cells of vascular tissue and they are thus also useful as antihypertensive agents and for the treatment of coronary vasospasm.

BRIEF DESCRIPTION OF THE INVENTION

According to the invention, there are provided novel 1,4-dihydropyridine derivatives of the formula:



(I)

wherein

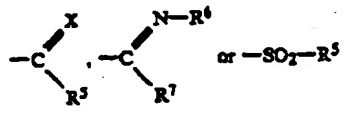
R is aryl or heteroaryl;

R^1 and R^2 are each independently C_1–C_4 alkyl or 2-methoxyethyl;

n is 2, 3 or 4;

R^3 is H, C_1–C_4 alkyl, $CH_2CO_2(C_1$–C_4 alkyl) or CH_2CN; and

R^4 is a group of the formula:



wherein

X is O or S;

R^5 is C_1–C_4 alkyl, NH_2, $NH(C_1$–C_4 alkyl), $NH(C_3$–C_6 cycloalkyl), $N(C_1$–C_4 alkyl)$_2$, $NHCH_2CO_2(C_1$–C_4 alkyl), $NHCH_2CONH_2$, $NHCH_2CO_2H$, $NH(CH_2)_2NH_2$, NH NH_2, $NHNRCO_2(C_1$–C_4 alkyl), NH-aryl, NHCO-aryl, or a heterocyclic, NH-heterocyclic or NHCO-heterocyclic group or when R^4 is $C(=O)R^5$, R^5 may be H or CF_3;

R^6 is H, CN, $CO_2(C_1$–C_4 alkyl), $CO(C_1$–C_4 alkyl), $SO_2(C_1$–C_4 alkyl), SO_2-aryl, SO_2NH_2, $SO_2N(C_1$–C_4 alkyl)$_2$, NO_2 or aryl; and

R^7 is NH_2, $NH(C_1$–C_4 alkyl), $NHCO(C_1$–C_4 alkyl), $NR(CM_2)_mN(C_1$–C_4 alkyl)$_2$ wherein m is 2 to 4 or a NH-heterocyclic group;

and their pharmaceutically acceptable acid addition salts.

The compounds of the formula (I) containing one or more asymmetric centres will exist as one or more pairs of enantiomers, and such pairs or individual isomers may be separable by physical methods, e.g. by fractional crystallisation of the free bases or suitable salts or chromatography of the free bases. The invention includes the separated pairs as well as mixtures thereof, as racemic mixtures or as separated d- and l-optically-active isomeric forms.

The pharmaceutically acceptable acid addition salts of the compounds of the formula (I) are those formed from acids which form non-toxic acid addition salts, for example the hydrochloride, hydrobromide, sulphate or bisulphate, phosphate or acid phosphate, acetate, citrate, fumarate, gluconate, lactate, maleate, succinate and tartrate salts.

The term "aryl" as used in this specification, includes phenyl and phenyl substituted by one or two substituents selected from nitro, halo, C_1–C_4 alkyl, C_1–C_4 alkoxy, hydroxy, trifluoromethyl, and cyano. It also includes 1- and 2-naphthyl.

The term "heteroaryl" as used in this specification means an aromatic heterocyclic group which may optionally be substituted and includes, for example, benzofuranyl; benzothienyl; pyridyl optionally monosubstituted by methyl, thiomethyl, halo or cyano; quinolyl; benzoxazolyl; benzthiazolyl; furyl; pyrimidinyl; thiazolyl; 2,1,3-benzoxadiazol-4-yl; 2,1,3-benzthiadiazol-4-yl; and thienyl optionally monosubstituted by halo or C_1–C_4 alkyl.

The term "heterocyclic group" used in connection with R^5 and R^7 means a 5 or 6 membered nitrogen, oxygen, or sulphur containing heterocyclic group which may be saturated or unsaturated and which may optionally include a further one or two nitrogen atoms in the ring and which may optionally be benzofused or substituted with for example, halo, C_1–C_4 alkyl, hydroxy, acetamido, carbamoyl oxo or $NR^{13}R^{14}$ groups where R^{13} and R^{14} are each independently H, C_1–C_4 alkyl or C_3–C_6 cycloalkyl or, together with the nitrogen atom to which they are attached, they form a 5 or 6 membered saturated heterocyclic ring optionally containing a further oxygen atom or NH or $N(C_1$–C_4 alkyl) group. Particularly suitable examples include pyridyl, pyrazinyl, hydroxypyridyl, dihydroxypyrimidinyl, piperidinyl, piperazinyl, 4-methyl-1-piperazinyl, morpholinyl, 1-imidazolidin-2-one, 2-furyl, thienyl, thiazolyl and quinolyl.

"Halo" means fluoro, chloro, bromo or iodo.

Alkyl and alkoxy groups having 3 or more carbon atoms can be straight or branched chain.

R is preferably 2-chlorophenyl or 2,3-dichlorophenyl. R^1 and R^2 are preferably CH_3 or C_2H_5, especially when R^1 is CH_3 and R^2 is C_2H_5. n is preferably 2. R^3 is preferably H or CH_3.

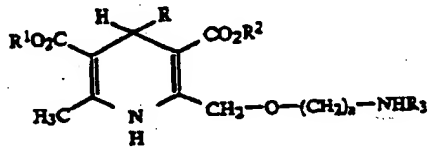
Preferred groups for R^4 are COR^5 where R^5 is H, $NHCH_3$, $NHCH_2CONH_2$ or 2-pyridon-5-yl; CSR^5 where R^5 is NH_2; $C(=NR^6)R^7$ where R^6 is CN and R^7 is $NHCH_3$; and SO_2R^5 where R^5 is NH_2, $NHCH_3$, NH

cyclopentyl, 2-thienyl, 8-quinolyl, or 2-(4-methylpiperazin-1-yl)pyrid-5-yl.

DETAILED DESCRIPTION OF THE INVENTION

The compounds of the formula (I) are prepared by a number of different processes according to the invention as follows:

(a) The ureas and thioureas of formula (I) wherein R^4 is $-C(=X)R^5$, X is O or S and R^5 is NH_2, $NH(C_1-C_4$ alkyl), $NH(C_3-C_6$ cycloalkyl), $NHCH_2CO_2(C_1-C_4$ alkyl), NH-aryl, NHCO-aryl, or a NH-heterocyclic or NHCO-heterocyclic group are prepared from an amine of the formula:



(II)

wherein R, R^1, R^2, R^3 and n are as previously defined by reacting with potassium cyanate or with an isocyanate or isothiocyanate of the formula:

$$R^8NCX \qquad \text{(III)}$$

wherein X is O or S and R^8 is C_1-C_4 lower alkyl, C_3-C_6 cycloalkyl, $CH_2CO_2(C_1-C_4$ alkyl), aryl, CO-aryl, or a heterocyclic or CO-heterocyclic group, respectively.

The reaction is simply performed by stirring the reactants together in a reaction-inert organic solvent at room temperature for several hours.

Compounds of the formula I wherein R^4 is $-C(=X)R^5$ and R^5 is $NHCH_2CONH_2$ or $NHCH_2CO_2H$ are readily prepared from the compound wherein R^5 is $NHCH_2CO_2(C_1-C_4$ alkyl) by reacting with concentrated ammonium hydroxide to yield the corresponding amides or by hydrolysing, for example with dilute sodium hydroxide, to yield the corresponding acids.

The urea derivatives wherein R^4 is $C(=X)R^5$, X is O and R^5 is NH_2 are prepared in a similar manner to that described above from the amine (II) but using potassium cyanate.

(b) The thiourea derivatives wherein R^4 is $-C(=S)R^5$ and R^5 is NH_2, $NH(C_1-C_4$ alkyl), $NH(C_3-C_6$ cycloalkyl) or $N(C_1-C_4$ alkyl)$_2$ are prepared by first reacting the amines of formula (II) with thiophosgene and reacting the resulting isothiocyanate intermediates with ammonia or with a (C_1-C_4) alkylamine, (C_3-C_8)cycloalkylamine or (di-C_1-C_4) alkylamine respectively.

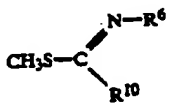
The reaction of the amine (II) and thiophosgene is conveniently performed by adding thiophosgene to a stirred solution of the amine in a mixture of water and methylene chloride in the presence of powdered calcium carbonate. After several hours at room temperature the organic layer containing the isothiocyanate intermediate is separated and the product isolated. This is then heated with ethanolic ammonia solution or with the appropriate amine to yield the thiourea product.

(c) In an alternative process, urea derivatives of formula (I) wherein R^4 is $C(=X)R^5$, X is O and R^5 is $NHCH_2CONH_2$, $NHCH_2CH_2NH_2$, NH-heterocyclic, $NHNH_2$ or $NHNHCO_2CH_2CH_3$ are prepared from an amine of formula (II) by first reacting with N,N'-carbonyldiimidazole and reacting the resulting imidazolylcarbonyl derivative with an amine or hydrazine derivative of formula $NH_2CH_2CONH_2$, $NH_2CH_2CH_2NH_2$, NH_2-heterocyclic, NH_2NH_2 or $NH_2NHCO_2CH_2CH_3$, respectively.

(d) The amides of formula (I) wherein R^4 is $C(=X)R^5$, X is O and R^5 is H, CF_3, C_1-C_4 alkyl, or a heterocyclic group are also prepared from the amines of formula (II) by reacting with an acid of formula R^9CO_2H, or with an anhydride, acid chloride or activated derivative thereof, wherein R^9 is H, CF_3, C_1-C_4 alkyl or a heterocyclic group respectively.

Thus, compounds wherein R^5 is H (R^4 is formyl) are prepared by a conventional formylation reaction, for example, using a mixture of formic acid and acetic anhydride. Compounds wherein R^5 is CF_3 or C_1-C_4 alkyl are similarly conveniently prepared using the appropriate acid anhydride in pyridine. The compounds wherein R^5 is a heterocyclic group are rather more conveniently prepared from the appropriate carboxy-substituted heterocyclic group by a coupling reaction, for example using a diimide condensing reagent such as 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide. Alternatively, in appropriate cases (for example when the heterocyclic group is a 1-piperidyl or 1-piperazinyl group) the heterocyclic group is initially reacted with phosgene to provide the N—COCl intermediate which is then reacted with the amine of formula II. All these reactions are quite conventional and conditions for their performance will be well known to those skilled in the art as will other possibilities and variations.

(e) Compounds of the formula (I) wherein R^4 is $-C(=N-R^6)R^7$ are again prepared from the amines of formula (II) by reacting with a compound of the formula:



(IV)

wherein R^6 is as previously defined and R^{10} is SCH_3, NR_2, or $NHCO(C_1-C_4$ alkyl) and, in the case where R^{10} is SCH_3, reacting the product with a C_1-C_4 alkylamine, dialkylamino-alkylamine, or heterocyclic amine.

The reaction between the amine (II) and the compound of formula (IV) is generally performed by heating the reactants together, in more or less equimolar proportions, in a reaction-inert organic solvent. A period of several hours heating under reflux in ethanol is generally found to be sufficient and the product is then simply isolated by removal of the solvent and purified by conventional procedures.

In the case where R^6 is H and R^{10} is NH_2 the compound of formula (IV) is S-methyl-isothiourea which is conveniently reacted as its sulphate to provide the unsubstituted guanidines of formula (I) wherein R^4 is $C(=NH)NH_2$. This can be further reacted with a sulphonyl halide to give the compounds where R^6 is $SO_2(C_1-C_4$ alkyl) or SO_2-aryl.

In the case where R^{10} is SCH_3 the resulting methylisothiourea is further reacted with an appropriate amine, usually by adding the reactants to ethanol at room temperature, typically for an overnight period, and the product is isolated by evaporation of the solvent and purified by conventional methods, e.g. by crystallisation. The methylisothiourea intermediate may also be generated from the thiourea of formula (I) (where R^3 is

H and R^4 is $C(=S)NH_2$) by reacting with methyl iodide. The resulting S-methylisothiouronium salt may then be reacted with the amine component as before.

(f) Compounds of the formula (I) wherein R^4 is $SO_2—R^5$ are again also prepared from the amines of formula (II) by reacting with sulphamide, or a sulphonyl chloride of the formula:

$$ClSO_2R^{11} \quad (V)$$

wherein R^{11} is C_1–C_4 lower alkyl, $NH(C_1$–C_4 alkyl), $NH(C_3$–C_6 cycloalkyl), $N(C_1$–C_4 alkyl)$_2$, NH-aryl, NHCO-aryl or a heterocyclic, NH heterocyclic or NHCO-heterocyclic group.

The reaction with sulphamide is typically achieved by heating the amine and sulphamide, in excess, under reflux in a reaction-inert organic solvent, e.g. dioxan. After a period of one or two hours the product is isolated and purified in a conventional manner. The reaction with a sulphonyl chloride of formula (V) is again performed in a conventional manner by adding the sulphonyl chloride to the amine in an inert organic solvent, e.g. dichloromethane, in the presence of an organic base, e.g. triethylamine. The reaction is generally complete after several hours at room temperature and the product is isolated and purified using conventional methods.

Preparation of the starting amines of formula (II) is described in the specification to our European patent application No. 89167. The various reactants of formula III, IV, V and R^9CO_2H, etc., are generally known compounds, either commercially available or they may be prepared by conventional methods in accordance with literature precedents.

The ability of the compounds of the invention to inhibit the movement of calcium into the cell is shown by their effectiveness in reducing the response of isolated heart tissue to an increase in calcium ion concentration in vitro. The test is performed by mounting spirally cut strips of rat aorta with one end fixed and the other attached to a force transducer. The tissue is immersed in a bath of physiological saline solution containing potassium ions at a concentration of 45 millimolar and no calcium. Calcium chloride is added to the bath with a pipette to give a final calcium ion concentration of 2 millimolar. The change in tension caused by the resulting contraction of the tissue is noted. The bath is drained and replaced with fresh saline solution and, after 45 minutes the test is repeated with the particular compound under test present in the saline solution. The concentration of compound required to reduce the response by 50% is recorded.

The antihypertensive activity of the compounds is also evaluated after oral administration by measuring the fall in blood pressure in spontaneously hypertensive rats or renally hypertensive dogs.

For administration to man in the curative or prophylactic treatment of cardiac conditions and hypertension, oral dosages of the compounds will generally be in the range of from 2–100 mg daily for an average adult patient (70 kg). Thus for a typical adult patient, individual tablets or capsules contain from 1 to 10 mg f active compound, in a suitable pharmaceutically acceptable vehicle or carrier. Dosages for intravenous administration would typically be within the range 1 to 10 mg per single dose as required. In practice the physician will determine the actual dosage which will be most suitable for an individual patient and it will vary with the age, weight and response of the particular patient. The above dosages are exemplary of the average case but there can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

For human use, the compounds of the formula (I) can be administered alone, but will generally be administered in admixture with a pharmaceutical carrier selected with regard to the intended route of administration and standard pharmaceutical practice. For example, they may be administered orally in the form of tablets containing such excipients as starch or lactose, or in capsules or ovules either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavouring or colouring agents. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood.

Thus in a further aspect the invention provides a pharmaceutical composition comprising a compound of the formula (I), or a pharmaceutically acceptable acid addition salt thereof, together with a pharmaceutically acceptable diluent or carrier.

The invention also provides a method of protecting the heart from the deleterious effects of ischaemia, which comprises administering an effective amount of a compound of the formula (I) or pharmaceutically acceptable acid addition salt thereof, or pharmaceutical composition as defined above.

The invention also includes a method of treating hypertension which comprises administering an antihypertensive amount of a compound of the formula (I) or pharmaceutically acceptable acid addition salt thereof, or pharmaceutical composition as defined above.

The following examples illustrate the invention:

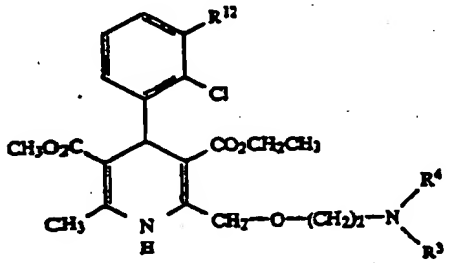
EXAMPLE 1

1-<2-{[4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-3-methylurea

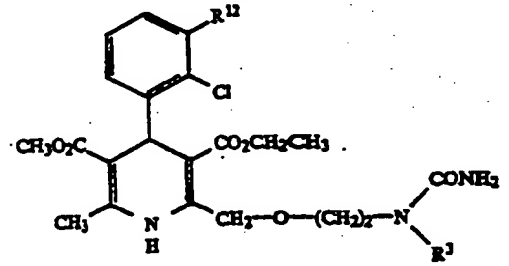
Methyl isocyanate (0.5 ml) was added to a solution of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.41 g) in dichloromethane (50 ml) and the mixture stirred at room temperature for 2 hours and then evaporated. The residue was triturated with diethyl ether and the solid collected, washed with diethyl ether, and dried to give the title compound (0.40 g), m.p. 75°–90° C. decomp. Found: C,56.81; H,6.14; N,9.21. $C_{22}H_{28}ClN_3O_6$ requires C,56.71; H,6.06; N,9.02%.

EXAMPLES 2–9

The following compounds were prepared by the method described in Example 1 from 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl or 2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine, or the appropriate substituted amine where R^3 is CH_3 or $CH_2CO_2CH_3$, and the appropriate isocyanate.



Example No.	R^{12}	R^3	R^4	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
2	H	CH_3	$CONHCH_3$	160–162	57.56 (57.55	6.45 6.30	8.55 8.76)
3	H	H	$CONHCH(CH_3)_2$	164	58.19 (58.35	6.58 6.53	8.60 8.51)
4	H	CH_3	$CONHCH_2CO_2CH_2CH_3$	116–120	56.18 (56.57	6.27 6.21	8.10 7.61)
5	H	CH_3	$CONHCOC_6H_5$	—	61.33 (61.10	5.52 5.66	7.48 7.37)
6	H	$CH_2CO_2CH_3$	$CONHCH_3$	174–176	55.95 (55.81	6.28 6.00	7.58 7.81)
7	H	H	$CONHC_6H_5$	156	57.51 (57.66	5.21 5.20	7.38 7.47)
8	Cl	$CH_2CO_2CH_3$	$CONHCH_3$	118–120	52.45 (52.22	5.45 5.41	7.34 7.12)
9	H	CH_2CN	$CONHCH_3$	113–115	56.78 (57.10	5.98 5.75	11.40 11.10)



Example No.	R^{12}	R^3	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
11	H	H	180–182	55.57 (55.81	5.79 5.76	9.20 9.30)
12	Cl	$CH_2CO_2CH_3$	176–178	51.15 (51.61	5.11 5.19	7.54 7.59)
13	H	CH_2CN	190–191	56.34 (56.27	5.80 5.50	10.99 11.42)

EXAMPLE 10

1-<2-{[4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-1-(methoxycarbonylmethyl)urea

A solution of potassium cyanate (0.24 g), methyl 2-<2-{[4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethylamino>acetate (0.96 g) and acetic acid (0.36 g) in dioxane (10 ml) and water (10 ml) was stirred at room temperature for 70 minutes and then evaporated. The residue was partitioned between water and ethyl acetate and the organic layer separated, dried (Na_2SO_4) and evaporated. The residue was triturated with ethyl acetate and the resulting solid collected and dried to give the title compound (0.77 g), m.p. 166°–169° C. Found: C,54.65; H,5.78; N,8.13. $C_{24}H_{30}ClN_3O_8$ requires C,55.01; H,5.77; N,8.02%.

EXAMPLES 11–13

The following compounds were prepared by the method described in Example 10 from potassium cyanate and the appropriate 2-(2-aminoethoxymethyl)-1,4-dihydropyridine or the corresponding substituted derivative of formula (I) wherein R^4 is hydrogen and R^3 is as defined below.

EXAMPLE 14

3-(Carbamoylmethyl)-1-<2-{[4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-1-methylurea

1-><2-{[4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-3-ethoxycarbonylmethyl-1-methylurea (0.44 g) was stirred in a mixture of ethanol (15 ml) and concentrated ammonium hydroxide (10 ml) for 24 hours and then evaporated. The residues was partitioned between chloroform and water and the organic layer dried (Na_2SO_4) and evaporated. The residue was triturated with diethyl ether/ethyl acetate and the resulting solid was collected, washed with diethyl

ether, and dried to give the title compound (0.23 g), m.p. 142°–143° C. Found: C,54.85; H,5.97; N,10.58. $C_{24}H_{31}ClN_4O_7$ requires C,55.12; H,5.97; N,10.71%.

EXAMPLE 15

3-Carboxymethyl-1-<2-{[4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-1-methylurea

1M Aqueous sodium hydroxide solution (2 ml) was added dropwise over 5 minutes to a stirred, ice-cooled solution of 1-<2-{[4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-3-ethoxycarbonylmethyl-1-methylurea (0.44 g) in dioxane (20 ml). The mixture was stirred at room temperature for 4 hours and then evaporated. The residue was dissolved in water, washed with diethyl ether, acidified with 2M hydrochloric acid, and extracted into chloroform. The chloroform layer was dried ($MgSO_4$) and evaporated. The residue was triturated with diethyl ether/ethyl acetate and the resulting solid was collected, washed with diethyl ether, and dried to give the title compound (0.10 g) as a gum. Found: C,54.76; H,5.77; N,8.14. $C_{24}H_{30}ClN_3O_8$ requires C,55.01; H,5.79; N,8.02%.

EXAMPLE 16

A. Preparation of 2-(4-aminobutoxy)methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine

A solution of 4-azidobutanol (6.8 g) in tetrahydrofuran (100 ml) was added dropwise over 30 minutes to a suspension of sodium hydride (5.4 g; 60% dispersion in oil) in tetrahydrofuran (100 ml). The mixture was stirred at room temperature for 30 minutes and then treated with a solution of ethyl 4-chloroacetoacetate (9.7 g) in tetrahydrofuran (150 ml) dropwise over 30 minutes. The mixture was stirred at room temperature for 16 hours, poured into water and the pH adjusted to 3–4 with 2M hydrochloric acid. The aqueous solution was extracted with ethyl acetate (3×200 ml) and the organic layer was dried ($MgSO_4$) and evaporated to give an oil which was taken up in acetonitrile and washed with petrol. The solvent was evaporated to dryness and the residue was chromatographed on silica eluting with a mixture of petrol and methylene chloride. Fractions containing the product were evaporated to give ethyl 4-(4-azidobutoxy)acetoacetate as a yellow oil (4.5 g). Methyl 3-aminocrotonate (2.2 g) and 2-chlorobenzaldehyde (2.7 g) in methanol (50 ml) were added and the mixture was heated under reflux for 5 hours and then evaporated. The residue was chromatographed on silica eluting with a mixture of petrol and ethyl acetate to give 2-(4-azidobutoxy)methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (5.9 g) as a yellow oil. The product was taken up in methanol (70 ml) and stirred at room temperature for 16 hours under 1 atmosphere of hydrogen in the presence of 5% palladium on calcium carbonate catalyst (2.0 g). The reaction mixture was filtered and concentrated. The residue was treated with a solution of fumaric acid (1.0 g) in methanol. The resulting precipitate was collected, treated with 2M ammonium hydroxide and extracted with methylene chloride (2×25 ml). The organic layer was washed with water, dried ($MgSO_4$), filtered and evaporated to give 2-(4-aminobutoxy)methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (2.2 g) as a yellow oil. The product was used in the next stage without further purification.

B.

4-(2-Chlor phenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-(4-[N-methylcarbamoyl]aminobutoxymethyl)-1,4-dihydropyridine, hemi-hydrate

Methyl isocyanate (0.25 ml) was added to a solution of 2-(4-aminobutoxy)methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.1 g) in dry methylene chloride (5 ml) and the mixture stirred at room temperature for 14 hours. The solvent was evaporated and the residue was chromatographed on silica eluting with methylene chloride containing 5% methanol by volume. Fractions containing the product were combined and evaporated to give an oil which crystallised when triturated with diethyl ether to afford the title compound (0.05 g), m.p. 126°–126.5° C. Found: C,57.4; H,6.6; N,8.5. $C_{24}H_{33}ClN_3O_6.0.5H_2O$ requires C,57.3; H,6.4; N,8.35%.

EXAMPLE 17

4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(thioureido)ethoxymethyl]-1,4-dihydropyridine

(A) Thiophosgene (0.9 ml) was added to a stirred mixture of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (4.08 g) and powdered $CaCO_3$ (3 g) in methylene chloride (25 ml) and water (35 ml). The mixture was stirred overnight at room temperature, filtered and partitioned between 2M hydrochloric acid and methylene chloride. The organic layer was washed with water, dried (Na_2CO_3), filtered and evaporated to give the isothiocyanate intermediate as a solid which was used directly for the next stage of the reaction without further purification.

(B) The isothiocyanate (4 g) was heated in ethanolic ammonia solution for 2½ hours. The precipitate was filtered and recrystallised from a mixture of ethanol and methylene chloride (5:1) to give the title compound, m.p. 203.5°–204.5° C. Found: C,53.3; H,5.5; N,8.6. $C_{21}H_{26}ClN_3O_5S$ requires C,53.8; H,5.6; N,8.9%.

EXAMPLE 18

4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[(2-N-methylthioureido)ethoxymethyl]-1,4-dihydropyridine was prepared by the method described in Example 17(B) but refluxing the isothiocyanate in ethanolic methylamine solution for 2 hours. The product solidified on trituration with diethyl ether, m.p. 138°–140° C. Found: C,54.24; H,5.79; N,8.72. $C_{22}H_{28}ClN_3O_5S$ requires C,54.82; H,5.86; N,8.72%.

EXAMPLE 19

4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(thi ureido)ethoxymethyl]-1,4-dihydropyridine

The title compound was prepared as described in Example 17 but starting with the corresponding 4-(2,3-dichlorophenyl)-1,4-dihydropyridine derivative, m.p. 198° C. F und: C,50.28; H,5.07; N,8.72. $C_{21}H_{25}Cl_2N_3O_5S$ requires C,50.20; H,5.02; N,8.30%.

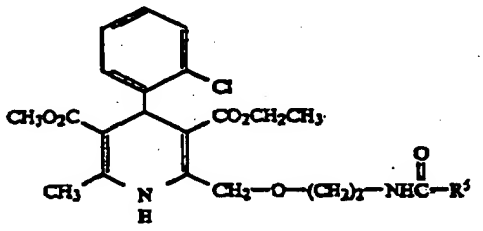
EXAMPLE 20

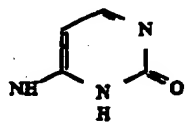
3-(Carbamoylmethyl)-1-<2-[[4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy]ethyl>urea

A mixture of 2-[2-[(1-imidazolylcarbonyl)amino]ethoxy]methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.98 g) (prepared from the 2-(2-aminoethoxymethyl)dihydropyridine derivative and N,N'-carbonyldiimidazole), glycinamide hydrochloride (0.22 g) and N-methylmorpholine (0.44 g) in acetonitrile (10 ml) was stirred at room temperature for 22 hours and then evaporated to dryness. The residue was chromatographed on silica eluting with hexane containing 30% by volume of dichloromethane, followed by dichloromethane containing from 0 to 1% by volume of methanol. Appropriate fractions were combined and evaporated to give the title compound (0.25 g). m.p. 114°–116° C. Found: C,53.95; H,5.85; N,10.80. $C_{23}H_{29}ClN_4O_7$ requires C,54.28; H,5.70; N,11.01%.

EXAMPLE 21–24

The following compounds were prepared by the method described in Example 20 from 2-[2-[(1-imidazolylcarbonyl)amino]ethoxy]methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine and the appropriate amine or hydrazine.



Example No.	R^5	m.p.(°C.)	Analysis % (Theoretical in brackets) C	H	N
21	$NHCH_2CH_2NH_2$	90 (decomp)	52.35 (52.30	6.31 5.81	9.10 9.04)
22		140–143	53.54 (53.24	5.04 5.32	11.84 12.42)
23	$NHNH_2$	144–146	54.02 (54.02	6.04 5.82	11.63 12.00)
24	$NHNHCO_2CH_2CH_3$	158–160	35.05 (35.48	5.87 5.80	10.65 10.40)

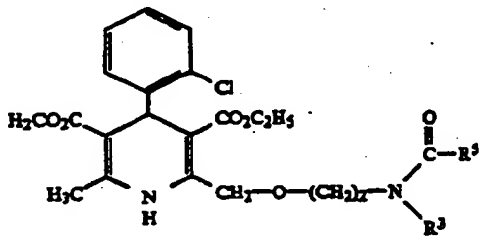
EXAMPLE 25

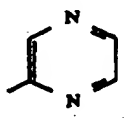
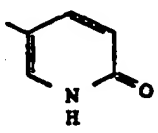
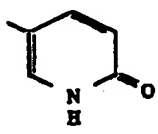
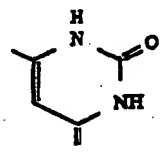
4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(3-pyridylcarbonylamino)ethoxymethyl]-1,4-dihydropyridine

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (0.42 g) was added to an ice-cooled solution of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.41 g), nicetinic acid (0.14 g) and 1-hydroxybenzotriazole hydrate (0.17 g) in dichloromethane (40 ml). The mixture was stirred with ice-cooling for 15 minutes, treated with N-methylmorpholine (0.61 g) and stirred at room temperature for 16 hours. The solution was then diluted with dichloromethane, washed successively with water, 2N hydrochloric acid, water, 10% aqueous sodium carbonate solution, and water, dried (Na_2SO_4) and evaporated. The residue was chromatographed on silica (t.l.c. grade, Merck Kieselgel 60H (Trade Mark) 8 g) eluting with dichloromethane plus 0–5% methanol. Appropriate fractions were combined and evaporated. The residue was triturated with diethyl ether and the resulting solid collected, washed with diethyl ether and dried to give the title compound (0.29 g); m.p. 81°–84° C. Found: C. 61.01; H, 5.48; N, 8.23. $C_{26}H_{28}ClN_3O_6$ requires C, 60.76; H, 5.49; N, 8.18%.

EXAMPLES 26–29

The following compounds were prepared by the method described in Example 25 from 2-(2-aminoethoxymethyl) or 2-(2-methylaminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-6-methoxycarbonyl-6-methyl-1,4-dihydropyridine and the appropriate carboxy substituted heterocyclic compound.



Example No.	R^3	R^5	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
26	H		117–118	58.54 (58.31	5.36 5.28	10.67 10.88)
27	H		125–130 decomp.	59.35 (58.92	5.78 5.33	7.70 7.93)
28	CH_3		74–81	58.15 (57.91	5.51 5.40	7.40 7.50)
29	CH_3		220–240 decomp.	56.13 (55.67	5.19 5.21	9.85 9.99)

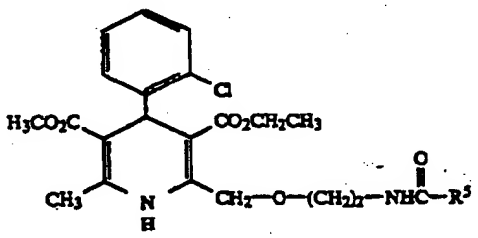
EXAMPLE 30

4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-2-[(N-formyl)aminoethoxy methyl]-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine

Formic acetic anhydride (15 ml; prepared by heating a mixture of formic acid (5 ml) and acetic anhydride (10 ml) at 50°–60° C. for 1 hour) was added over 10 minutes to a stirred, ice-cooled solution of 2-(2-aminoethoxymethyl)-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (4.4 g) in tetrahydrofuran (30 ml). The mixture was stirred at room temperature for 1.5 hours and then evaporated. The residue was dissolved in dichloromethane and the solution washed with 10% aqueous sodium carbonate solution, dried (Na_2SO_4), and evaporated. The residual solid was collected, washed with diethyl ether and dried to give the title compound as a hemihydrate (4.0 g), m.p. 178°–181° C. Found: C, 52.69; H, 5.05; N, 6.06. $C_{21}H_{24}Cl_2N_2O_6.0.5H_2O$ requires C, 52.51; H, 5.25; N, 5.83%.

EXAMPLES 31–33

The following examples were prepared by the method described in Example 30 from the corresponding 4-(2-chlorophenyl)-1,4-dihydropyridine derivative using formic/acetic anhydride, acetic anhydride or trifluoroacetic anhydride respectively:



Example No.	R^5	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
31	H	164–166	56.51 (56.57	5.82 5.88	6.04 6.29)
			This compound is a hemihydrate		
32	CH_3	97–99	59.00 (58.60	6.16 6.04	6.24 6.21)
33	CF_3	141–143	52.41 (52.33	4.83 4.79	5.50 5.56)

EXAMPLE 34

4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(4-methylpiperazin-1-ylcarbonylamino)ethoxymethyl]-1,4-dihydropyridine

A solution of N-methylpiperazine in toluene (5 ml) and triethylamine (1 ml) was added dropwise to a 12.5% (by weight) solution of phosgene in toluene (7.5 ml) at −30° C. The solution was allowed to warm to room temperature over 1.5 hours and then purged with nitrogen to remove excess phosgene. 2-(2-Aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine was then added and the mixture allowed to stir at room temperature for 17 hours. After evaporation to dryness, the resultant oil was partitioned between 5% aqueous sodium carbonate and diethyl ether. The combined organic liquors were dried ($MgSO_4$), filtered and evaporated to give 0.7 g of a colourless oil. This oil was chromatographed on Kieselgel 60 (Trade Mark) (3 g), eluting with ethyl acetate to give 0.2 g of a white solid. Crystallisation from ethyl acetate afforded the pure title compound (0.13 g), m.p. 78°–80° C. Found: C, 58.36; H, 6.59; N, 10.47%. $C_{26}H_{35}ClN_4O_6$ requires C, 58.35; H, 6.64; N, 10.41%.

EXAMPLE 35

4-(2-Chlorophenyl)-3-ethoxycarbonyl-2-[2-(imidazolidin-2-on-1-ylcarbonylamino)ethoxymethyl]-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine

To a solution of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (1.0 g) in a mixture of chloroform (dried over alumina) (20 ml) and triethylamine (2 ml) was added imidazolidin-2-on-1-yl carbonyl chloride (0.36 g) in one portion and the mixture stirred at room temperature for 18 hours. After evaporation to dryness, the resultant oil was partitioned between 5% aqueous sodium carbonate and methylene chloride. The combined organic liquors were dried ($MgSO_4$), filtered and evaporated to give 0.7 g of a yellow oil which crystallised from diisopropyl ether on standing to give pure title compound, (0.4 g), m.p. 145° C. Found: C, 54.94; H, 5.62; N, 10.62. $C_{24}H_{29}ClN_4O_7$ requires C, 55.33; H, 5.61; N, 10.76%.

EXAMPLE 36

3-<2-{[4-(2-Chlorophenyl-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-2-cyano-1-methylguanidine

(A) A solution of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.82 g) and di(methylthio)-methylidinoiminonitrile (0.29 g) in ethanol (50 ml) was heated under reflux for 2 hours and then evaporated. The residue was triturated with diethyl ether and the resulting solid collected, washed with ether, and dried to give 1->2-{[4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-3-cyano-2-methylisothiourea (0.96 g), m.p. 177°–179° C. Found: C, 54.34; H, 5.42; N, 11.18. $C_{23}H_{27}ClN_4O_5S$ requires C, 54.49; H, 5.37; N, 11.05%.

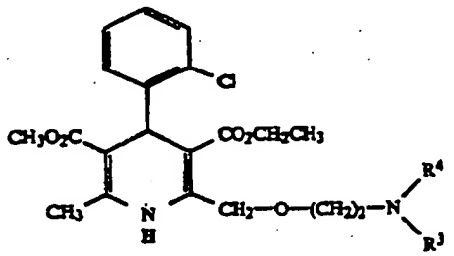
(B) The product from (A) (0.40 g) in 33% (by weight) ethanolic methylamine solution (10 ml) was stirred for 18 hours and then evaporated. The residue was recrystallised from ethanol to give the title compound (0.25 g), m.p. 188°–190° C. Found: C, 56.66; H, 5.53; N, 14.08, $C_{23}H_{28}ClN_2O_5$ requires C, 56.38; H, 5.76; N, 14.29%.

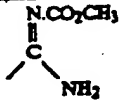
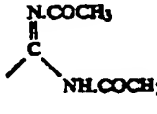
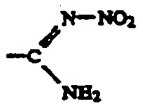
EXAMPLE 37

2-Cyano-3-<2-{[4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-1-methylguanidine was prepared by the method described in Example 36B from 3-cyano-1-<2-{[4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-2-methylisothiourea. The product was btained as a monohydrate which had m.p. 175° C. Found: C, 50.62; H, 5.12; N, 12.81. $C_{23}H_{27}Cl_2N_5O_5.H_2O$ requires C, 50.92; H, 5.38; N, 12.91%.

EXAMPLES 38–40

The following compounds were prepared by the method described in Example 36(A) from 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine and the appropriate compound of formula (IV).



Example No.	R^3	R^4	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
38	H		115–116	53.95 (54.27	5.74 5.74	11.19 11.01)
39	H		146–147	56.00 (56.12	5.86 5.84	10.15 10.47)
40	H		122–124	50.83 (50.96	6.10 5.91	12.66 12.92)

EXAMPLE 41

1-<2-{[4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-2-cyano-3-(2-dimethylamino)ethylguanidine was prepared by the method described in Example 36(B) but using ethanolic N,N-dimethylethylenediamine instead of ethanolic methylamine. The product had m.p. 202°–204° C. decomp. Found: C, 56.67; H, 6.51; N, 15.58. $C_{26}H_{35}ClN_6O_5$ requires C, 57.08; H, 6.45; N, 15.36.

EXAMPLE 42

4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-{2-[N-(2-thiazolyl)guanidino]ethoxymethyl}-1,4-dihydropyridine hydrate

(A) 4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(thioureido)ethoxymethyl]-1,4-dihydropyridine (4.2 g) was dissolved in a mixture of methanol (25 ml) and methylene chloride (25 ml) and stirred at room temperature. Methyl iodide (1.2 g) was added and the solution stirred for 40 hours. After evaporation to dryness, trituration with diethyl ether gave the intermediate S-methylisothiouronium hydriodide as a solid which was used directly for the next stage of the reaction without further purification.

(B) The S-methylisothiouronium hydriodide (3.3 g) and 2-aminothiazole (0.64 g) were suspended in a mixture of n-butanol (25 ml) and triethylamine (3.3 g) and the mixture stirred at reflux for 5 hours. After evaporation to dryness, the residual oil was partitioned between 5% aqueous sodium bicarbonate (50 ml) and methylene chloride (3×75 ml). The combined organic extracts were dried ($MgSO_4$), filtered and evaporated to give a dark brown oil (4.2 g). The residual oil was chromatographed on silica eluting with toluene containing an increasing proportion of ethylacetate. The product was finally crystallised from toluene to give the title compound, m.p. 116°–119° C. Found: C, 49.21; H, 4.67; N, 12.15. $C_{24}H_{27}Cl_2N_5O_5S.H_2O$. requires C, 49.15; H, 4.98; N, 11.94%.

EXAMPLE 43

4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-2-[2-(guanidino)ethoxymethyl]-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine

2-(2-Aminoethoxymethyl)-4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (5.0 g) and S-methylisothiourea sulphate (3.45 g) was suspended in n-propanol (120 ml) and triethylamine (30 ml) and refluxed for 17 hours. After evaporation to dryness, the residual oil was partitioned between aqueous sodium bicarbonate and methylene chloride. The organic layer was dried ($MgSO_4$), filtered and evaporated. The resultant beige solid was triturated with hexane to give the title compound (4.1 g), N.M.R. ($CDCl_3$, 60 MHz): 3H 1.15(t), 3H 3.55(s), 4H 3.7(m), 2H 4.0(q), 2H 4.7(s), 1H 5.45(s), 8H 7.25(m).

EXAMPLE 44

4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-{2-[N-(N'-phenyl-sulphonyl)-guanidinyl]ethoxymethyl}-1,4-dihydropyridine

4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-2-[2-(N-guanidino)ethoxymethyl]-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (1.19 g) was added to 10% aqueous sodium hydroxide solution (40 ml), followed by benzenesulphonyl chloride (0.9 g). The mixture was shaken vigorously for 15 minutes and stirred at room temperature for a further 1 hour. The resultant mixture was extracted with diethyl ether (2×25 ml) and then methylene chloride (3×75 ml). The combined methylene chloride liquors were dried ($MgSO_4$), filtered and evaporated and the residual yellow solid chromatographed on silica eluting with 50% by volume ethyl acetate in toluene. Crystallisation of the product from petroleum ether (b.p. 100°–120° C.) and ethyl acetate afforded the title compound, 0.19 g, m.p. 153°–154°. Found: C, 52.22; H, 5.02; N, 8.79. $C_{27}H_{30}Cl_2N_4O_7S$ requires C, 51.84; H, 4.83; N, 8.96%.

EXAMPLE 45

4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-2-{2-[(N-methanesulphonyl)-N-methylamino]ethoxymethyl}-6-methyl-1,4-dihydropyridine

Methanesulphonyl chloride (0.144 g) was added to a solution of 4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(methylamino)ethoxymethyl]-1,4-dihydropyridine (0.50 g) and triethylamine (0.126 g) in dichloromethane (15 ml). The mixture was stirred at room temperature for 1 hour, poured into ice-water and the layers separated. The organic layer was washed with water, dried (Na_2SO_4) and evaporated. Scratching the residual oil afforded a solid which was recrystallised from methanol to give the title compound (0.33 g), m.p. 93°–95° C. Found: C, 53.32; H,

5.97; N, 5.64. $C_{22}H_{29}ClN_2O_7S$ requires C, 53.32; H, 5.92; N, 5.71%.

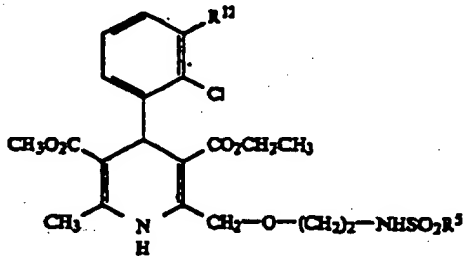
EXAMPLE 46

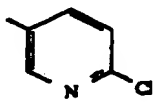
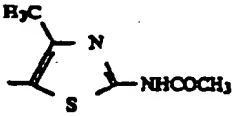
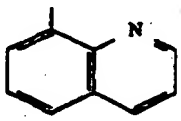
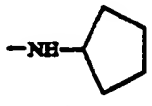
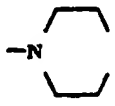
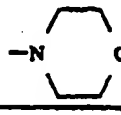
4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-{2-[(2-chloro-5-pyridyl)sulphonamido]ethoxymethyl}-1,4-dihydropyridine

A solution of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (409 mg) and triethylamine (202 mg) in dry dichloromethane (20 ml) was stirred under nitrogen and cooled in an ice-bath while a solution of 2-chloro-5-pyridinesulphonyl chloride (212 mg) in dry dichloromethane (10 ml) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirring continued for a further 3 hours. The solvent was evaporated and the residue chromatographed on silica, eluting with dichloromethane. Appropriate fractions were combined and evaporated and the residue recrystallised from ethyl acetate to give the title compound (320 mg), m.p. 140°–140.5° C. Found: C, 51.27; H, 4.63; N, 7.25. $C_{25}H_{27}Cl_2N_3O_7$ requires C, 51.37; H, 4.66; N, 7.19%.

EXAMPLES 47–53

The following compounds were prepared following the general procedures described in Examples 45 or 46 starting with 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl or 2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine and the appropriate sulphonyl chloride.



Example No.	R^5	R^{12}	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
47		Cl	195.5–198	48.34 (48.51	4.32 4.23	6.88 6.79)
48		Cl	199–200.5	48.85 (48.89	4.40 4.45	4.29 4.75)
49		Cl	153–156	47.46 (47.20	4.89 4.57	8.81 8.67)
50		Cl	160–162	54.85 (54.89	4.51 4.61	6.62 6.62)
51		Cl	151–152	51.05 (50.85	5.63 5.63	7.41 7.12)
52		H	155–157	51.56 (51.65	5.9 5.8	7.5 7.8)
53		Cl	169–170	48.9 (48.65	5.2 5.3	6.9 7.1)

EXAMPLE 54

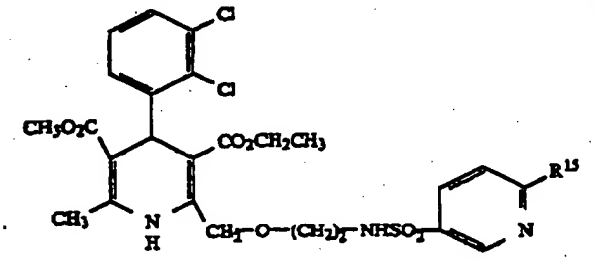
4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-{2-[(2-(4-morpholino-5-pyridyl)-sulphonamido]ethoxymethyl}-1,4-dihydropyridine

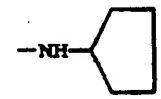
A solution of 4-(2,3-dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-{2-[(2-chloro-5-pyridyl)sulphonamido]-ethoxymethyl}-1,4-dihydropyridine (200 mg) in morpholine (2 ml) was heated on a steam bath for 6 hours.

The reaction mixture was evaporated to dryness and the residue partitioned between dichloromethane and water. The organic layer was separated, washed with water, dried over anhydrous magnesium sulphate and evaporated to give an orange gum. Chromatography on silica eluting with dichloromethane followed by dichloromethane containing 2% by volume of methanol gave the title compound (125 mg) which was recrystallised from ethyl acetate, m.p. 172.5°–173° C. Found: C, 52.04; H, 5.10; N, 8.48. $C_{29}H_{34}Cl_2N_4O_8$ requires C, 52.02; H, 5.12; N, 8.37%.

EXAMPLES 55 AND 56

The following compounds were prepared in a similar manner to Example 54 but using cyclopentylamine or N-methylpiperazine, respectively instead of morpholine.



Example No.	R^{15}	m.p. (°C.)	Analysis % (Theoretical in brackets) C	H	N
55		180–182	53.96 (53.97	5.52 5.44	8.21 8.39)
56		172–174.5	52.72 (52.78	5.42 5.46	10.08 10.26)

EXAMPLE 57

2-{[4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethylsulphamide

A solution of 2-(2-aminoethoxy)methyl-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.82 g) and sulphamide (0.96 g) in dioxane (30 ml) was heated under reflux for 70 minutes and then evaporated. The residue was partitioned between ethyl acetate and water and the organic layer dried (Na_2SO_4) and evaporated. The residue was triturated with diethyl ether and the resulting solid collected, washed with diethyl ether and dried to give the title compound (0.75 g), m.p. 150°–152° C. F und: C, 49.33; H, 5.35; N, 8.93. $C_{20}H_{26}ClN_3O_7S$ requires C, 49.23; H, 5.37; N, 8.61%.

EXAMPLE 58

2-{[4-(2,3-Dichlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethylsulphamide

This compound was prepared as described above starting with the corresponding 4-(2,3-dichlorophenyl)-dihydropyridine. m.p. 89°–90° C. Found: C, 45.57; H, 4.89; N, 7.86. $C_{20}H_{25}Cl_2N_3O_7S$ requires C, 45.98; H, 4.79; N, 8.04.

EXAMPLE 59

1-<2-{[4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridin-2-yl]methoxy}ethyl>-1-methyl sulphamide was prepared by the method described in Example 57 from 4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(N-methylamino)ethoxy]methyl-1,4-dihydropyridine. The product had m.p. 117°–120° C. Found: C, 50.66; H, 5.69; N, 8.22. $C_{21}H_{28}ClN_3O_7S$ requires C, 50.25; H, 5.62; N, 8.37%.

EXAMPLE 60

4-(2-Chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-2-[2-(4-methyl-1-piperazinylsulphonylamino)ethoxymethyl]-1,4-dihydropyridine

2-(2-Aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.5 g) was dissolved in a mixture of chloroform (dried over alumina) (20 ml) and triethylamine (2 ml) and the mixture stirred at room temperature. 4-Methyl-1-piperazinylsulphonyl chloride (0.25 g) was added in one portion and the mixture stirred at room temperature for 17 hours. After evaporation to dryness, the resultant oil was partitioned between 5% aqueous sodium carbonate and methylene chloride. The organic layer was dried ($MgSO_4$), filtered and evaporated to give 0.3 g of a colourless oil, which was dissolved in diisopropyl ether (15 ml) and kept in a refrigerator for 14 days. The resultant crystals were collected by filtration to afford the title compound (0.1 g), m.p. 137°–139° C. Found: C, 52.58; H, 6.18; N, 9.81%. $C_{25}H_{35}ClN_4O_7S$ requires C, 53.03; H, 6.14; N, 9.20%.

EXAMPLE 61

4-(2-Chlorophenyl)-3-ethoxycarbonyl-2-[2-(2-furoylaminosulphonylamino)ethoxymethyl]-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine

To a solution of 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-3-ethoxycarbonyl-5-methoxycarbonyl-6-methyl-1,4-dihydropyridine (0.5 g) in chloroform (dried over alumina) and triethylamine (2 ml) was added 2-furoylsulphamoyl chloride in one portion and the mixture stirred at room temperature for 17 hours. After evaporation to dryness, the resultant oil was partitioned between 5% aqueous sodium bicarbonate and methylene chloride. The combined organic liquors were dried ($MgSO_4$), filtered and evaporated to give 0.35 g of solid which was crystallised from diisopropyl ether to give pure title compound (0.2 g), m.p. 138° C. Found: C, 51.30; H, 5.23; N, 7.41. $C_{25}H_{28}ClN_3O_9S$ requires C, 51.59; H, 4.85; N, 7.22%.

EXAMPLE 62

Tablets are compounded from the following ingredients:

	mg/tablet
Product of any one of Examples	10
Dicalcium phosphate	120
Magnesium stearate	1.8
Sodium lauryl sulphate	0.2

EXAMPLE 63

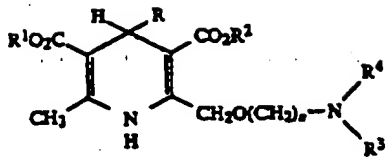
Capsules are compounded from the following ingredients:

	mg/capsule
Product of any one of Examples	10
Maize starch	127
Cellulose (microcrystalline)	127
Magnesium stearate	5.4
Sodium lauryl sulphate	0.6

The ingredients are thoroughly blended, then filled into hard gelatine capsules of the appropriate size to contain the ingredients.

We claim:

1. A 1,4-dihydropyridine compound having the formula:



(I)

wherein R is 2,3-dichlorophenyl;
R^1 and R^2 are each independently C_1–C_4 alkyl;
n is 2; R^3 is H or C_1–C_4 alkyl; and
R_4 is a group of the formula

$$-SO_2R^5$$

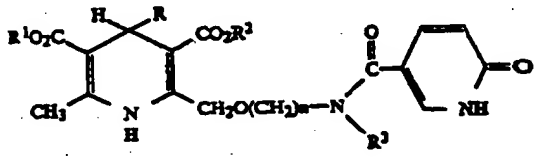
wherein
R^5 is 2-chloropyrid-5-yl or 2-(4-methylpiperazin-1-yl)pyrid-5-yl and a pharmaceutically acceptable acid addition salt thereof.

2. A compound according to claim 1 wherein R^1 is CH_3 and R^2 is C_2H_5.

3. A compound according to claim 2 wherein R^3 is H or CH_3.

4. A compound according to claim 3 wherein R^4 is SO_2R^5 where R^5 is 2-(4-methylpiperazin-1-yl)pyrid-5-yl.

5. A 1,4-dihydropyridine compound having the formula



wherein R is 2-chlorophenyl;
R^1 and R^2 are each independently C_1–C_4 alkyl; n is 2; and R^3 is H or C_1–C_4alkyl.

6. A method for treating hypertension in a mammal comprising administering to said mammal an antihypertensive effective amount of a compound according to claim 1 or 5.

* * * * *

AST 4

United States Patent [19]

Davison et al.

[11] **Patent Number: 4,879,303**

[45] **Date of Patent: Nov. 7, 1989**

[54] **PHARMACEUTICALLY ACCEPTABLE SALTS**

[75] Inventors: **Edward Davison, Margate; James I. Wells, Canterbury, both of England**

[73] Assignee: **Pfizer Inc., New York, N.Y.**

[21] Appl. No.: **256,938**

[22] Filed: **Oct. 13, 1988**

Related U.S. Application Data

[63] Continuation of Ser. No. 30,658, Mar. 25, 1987, abandoned.

[30] **Foreign Application Priority Data**

Apr. 4, 1986 [GB] United Kingdom 8608335

[51] Int. Cl.[4] C07D 211/86; A61K 31/455

[52] U.S. Cl. 514/356; 546/321

[58] Field of Search 514/356; 546/321

[56] **References Cited**

U.S. PATENT DOCUMENTS

3,816,612	6/1974	Schmidt et al.	425/45
4,032,637	6/1977	Spiegel et al.	514/224

OTHER PUBLICATIONS

Berge et al., Jour. of Pharmaceutical Science, Jan. 1977, vol. 66, No. 1.

Primary Examiner—Jane T. Fan

Attorney, Agent, or Firm—Peter C. Richardson; J. Trevor Lumb; James M. McMamus

[57] **ABSTRACT**

Improved pharmaceutical salts of amlodipine, particularly the besylate salt, and pharmaceutical compositions thereof. These salts find utility as anti-ischaemic and anti-hypertensive agents.

11 Claims, No Drawings

PHARMACEUTICALLY ACCEPTABLE SALTS

This application is a continuation application of co-pending application Ser. No. 07/030,658, filed Mar. 25, 1987, now abandoned.

BACKGROUND OF THE INVENTION

The present invention relates to improved pharmaceutical salts of amlodipine and pharmaceutical compositions thereof.

The compound amlodipine (3-ethyl 5-methyl 2-(2-aminoethoxymethyl)-4-(2-chlorophenyl)-1,4-dihydro-6-methylpyridine-3,5-dicarboxylate) is a potent and long acting calcium channel blocker having utility as an anti-ischaemic and anti-hypertensive agent.

European Patent Application Publication No. 89167 and U.S. Pat. No. 4,572,909 disclose several different pharmaceutically acceptable salt forms of amlodipine. In particular, the pharmaceutically acceptable acid addition salts are said to be those formed from acids which form non-toxic acid anions such as the hydrochloride, hydrobromide, sulphate, phosphate or acid phosphate, acetate, maleate, fumarate, lactate, tartrate, citrate and gluconate salts. Of these salts the maleate is disclosed as being particularly preferred.

SUMMARY OF THE INVENTION

It has now unexpectedly been found that the benzene sulphonate salt (hereinafter referred to as the besylate salt) has a number of advantages over the known salts of amlodipine and, additionally, has unexpectedly been found to have a unique combination of good formulation properties which make it particularly suitable for the preparation of pharmaceutical formulations of amlodipine.

Thus according to the present invention there is provided the besylate salt of amlodipine.

In a further aspect the invention provides a pharmaceutical composition of the besylate salt of amlodipine together with a pharmaceutically acceptable diluent or carrier.

The invention further provides a tablet formulation comprising the besylate salt of amlodipine in admixture with excipients. A preferred formulation includes the besylate salt, a compression aid such as microcrystalline cellulose, an additive to provide sheen to the table such as anhydrous dibasic calcium phosphate, a disintegrant such as sodium starch glycollate and a lubricant such as magnesium stearate.

In addition the invention provides a capsule formulation comprising the besylate salt of amlodipine in admixture with excipients. A preferred formulation includes the besylate salt, an inert diluent; a dried disintegrant and a lubricant as described above.

The invention further provides the besylate salt of amlodipine in sterile aqueous solution for parenteral administration. Preferably such solution contains from 10 to 40% by volume of propylene glycol and preferably also sufficient sodium chloride to avoid haemolysis, e.g. about 1% w/v.

The invention also provides the besylate salt of amlodipine for use in treating ischaemic heart disease, especially angina, or hypertension, in a human being.

The invention also provides a process for preparing the besylate salt of amlodipine by reacting amlodipine base with a solution of benzenesulphonic acid in an inert solvent and recovering the besylate salt of amlodipine.

The preferred inert solvent is industrial methylated spirit.

DETAILED DESCRIPTION OF THE INVENTION

Although amlodipine is effective as the free base, in practice it is best administered in the form of a salt of a pharmaceutically acceptable acid. In order to be suitable for this purpose the pharmaceutically acceptable salt must satisfy the following four physiochemical criteria: (1) good solubility; (2) good stability; (3) non-hydroscopicity; (4) processability for tablet formulation, etc.

It has been found that whilst many of the salts outlined above satisfy some of these criteria, none satisfy them all and even the preferred maleate, whilst exhibiting excellent solubility tends to break-down in solution after a few weeks. Consequently a range of pharmaceutically acceptable salts of amlodipine has been made and evaluated using these criteria:

1. Generally, it is known in the art that a good aqueous solubility is necessary for good bioavailability. Usually a solubility of greater than 1 mg ml^{-1} at pH 1–7.5 is sought although higher solubilities are required to formulate injections. In addition, salts which provide solutions having a pH close to that of blood (7.4) are preferred because they are readily biocompatible and can easily be buffered to the required pH range without altering their solubility.

As can be seen from the following comparative data the besylate salt of amlodipine exhibits good solubility characteristics, compared with other salts.

TABLE 1

Salt	solubility mg ml^{-1}	pH at saturation
Benzene sulphonate (besylate)	4.6	6.6
Toluene sulphonate (tosylate)	0.9	5.9
Methane sulphonate (mesylate)	25	3.1
Succinate	4.4	4.9
Salicylate	1.0	7.0
Maleate	4.5	4.8
Acetate	50	6.6
Hydrochloride	50	3.5

2. Good stability in the solid state is very important for tablets and capsules, whilst good stability in solution is required for an aqueous injection.

In order to screen for chemical stability, each of the salts was blended in a powder vehicle and formed into tablets or capsules. In the case of tablets the vehicle comprised microcrystalline cellulose in 50:50 combination with anhydrous dibasic calcium phosphate. In the case of capsules the vehicles comprised mannitol in 4:1 combination with dried maize starch. These were then stored in sealed vials at 50° and 75° C. for up to three weeks. The drug and any breakdown products were extracted with methanol:chloroform (50:50) and separated on silica tlc plates using a variety of solvent systems.

The results were compared and the salts ranked according to the number and amount of breakdown product produced.

By comparing the results the following rank order emerges with besylate being the most stable salt and hydrochloride the least stable.

Salt	Stability
Besylate	most stable
Mesylate	↓
Tosylate	↓
Succinate	↓
Salicylate	↓
Maleate	↓
Acetate	↓
Hydrochloride	unstable

3. In order to provide stable formulations it is desirable to have a non-hygroscopic salt. In the solid state where drug content is high, absorbed films of moisture can act as a vector for hydrolysis and chemical breakdown. It is the hygroscopic nature of a drug or its salt which contributes to the free moisture which is normally responsible for instability.

Only the maleate, tosylate and besylate salts do not pick up any moisture when exposed to 75% relative humidity at 37° C. for 24 hours. Even when exposed to 95% relative humidity at 30° C. for 3 days both the besylate and maleate remain anhydrous whilst the tosylate formed the dihydrate salt. Therefore the besylate salt can be considered to be non-hygroscopic and thus provides stale formulations while minimising the risk of intrinsic chemical breakdown.

4. The final characteristic of an acceptable salt to be considered is the processability, i.e. the compression properties and also the ability not to stick or adhere to the tablet making machinery.

For high dose formulations, good compressibility is very important to make elegant tablets. With lower dose tablets the need for good compressibility can be eliminated to a certain extent by the use of suitable diluting excipients called compression aids. Microcrystalline cellulose is a commonly used compression aid. However whatever the dose the adhesion of the drug to the punches of the tablet machine must be avoided. When drug accumulates on the punch surfaces this causes the tablet surface to become pitted and therefore unacceptable. Also sticking of the drug in this way results in high ejection forces when removing the tablet from the machine. In practice it is possible to reduce sticking by wet-massing, careful selection of excipients and the use of high levels of anti-adherents, e.g. magnesium stearate. However selection of a salt with good anti-adhesion properties minimises these problems.

In order to compare the stickiness of the various salts of amlodipine the following procedure was carried out using conventional tablet making machinery: fifty tablets containing calcium sulphate dihydrate, microcrystalline cellulose and amlodipine besylate were made (47.5:47.5:5), the material sticking to the tablet punch was then extracted using methanol and the amount measured spectrometrically. This procedure was then repeated for runs of 100, 150, 200, 250 and 300 tables. After each run the amount of material sticking to the tablet punch was measured after extraction with methanol. The values are plotted and an average value calculated from the slope of the line produced.

This same procedure was then repeated for each of the salts of amlodipine. The amount of amlodipine measured as sticking to the tablet punch is shown in Table 2 for each salt and relative to the maleate salt.

TABLE 2

Salt	Stickiness: g Amlodipine cm^{-2} $tablet^{-1}$	Stickiness: Relative to maleate
Mesylate	1.16	58%
Besylate	1.17	59
Tosylate	1.95	98
Maleate	1.98	100
Free base	2.02	102
Succinate	2.39	121
Hydrochloride	2.51	127
Salicylate	2.85	144

Clearly the besylate has superior anti-adhesion properties to the maleate. Whilst the mesylate also shows good processability it tends to be isolated as the anhydride but this equilibrates to the monohydrate leading to variable composition after manufacture which makes it unacceptable for use in tablets.

Thus the besylate salt of amlodipine shows a unique combination of good solubility, good stability, non-hygroscopicity and good processability which makes it outstandingly suitable for the preparation of pharmaceutical formulations of amlodipine.

In order that the present invention be more readily understood, reference is now made to the following Examples.

EXAMPLE 1

Preparation of Besylate Salt of Amlodipine

Amlodipine base (65.6 g, 0.161 mols) was slurried in industrial methylated spirit (326.4 ml) and cooled to 5° C. Benzenesulphonic acid (26.2 g, 0.168 mols) was dissolved in industrial methylated spirit (65.6 ml) at 5° C. and added to the slurry of the base. The resulting slurry was then granulated, filtered and washed with 2 volumes of industrial methylated spirit (65.6 ml). The damp solid was slurried at 5° C. for 1 hr in industrial methylated spirit (327.6 ml), filtered, washed with 2 volumes of industrial methylated spirit (65.6 ml) and dried under vacuum at 55° C. for 24 hours. A yield of 6.5 g (83.8%) was obtained with the following analysis.

	Melting Point 201.0° C.		
Analysis %	C	H	N
Calc.	55.07	5.51	4.94
Found	54.91	5.46	4.93

EXAMPLE 2

Formulation of Tablets Containing Besylate Salt of Amlodipine

Amlodipine besylate was blended with sodium starch glycollate and anhydrous dibasic calcium phosphate for 5 minutes. This mixture was then sieved, reblended and sieved again followed by blending with microcrystalline cellulose. The resultant mixture was then sieved again and blended for a further 10 minutes. Finally magnesium stearate was added and the whole mixture blended for 5 minutes. The blend was then pressed into tablets using conventional tablet making machinery.

TABLE 3

TABLET COMPOSITIONS

Besylate salt (mg)	Microcrystalline cellulose (mg)	Anhydrous dibasic calcium phosphate (mg)	Sodium starch glycollate (mg)	Magnesium stearate (mg)
1.736	63.514	31.750	2.00	1.00
3.472	62.028	31.500	2.00	1.00
6.944	124.056	63.000	4.00	2.00
13.889	248.111	126.000	8.00	4.00

This method was used to make tablets containing different concentrations of the amlodipine besylate salt as shown in table 3.

EXAMPLE 3

Formulation of Capsules Containing Besylate Salt of Amlodipine

Microcrystalline cellulose and dried maize starch were preblended. The besylate salt of amlodipine was then mixed with some of this preblend and then sieved. The remainder of the preblend was then added and mixed for 10 minutes. This was then sieved again and mixed for a further 5 minutes.

This method was used to make mixtures containing different concentrations of the amlodipine besylate salt as shown in Table 4 and the mixtures were then filled into capsules of appropriate size.

TABLE 4

CAPSULE COMPOSITIONS

Besylate salt (mg)	Microcrystalline cellulose (mg)	Dried Maize starch (mg)	Magnesium stearate (mg)	Total Capsule weight (mg)
1.736	38.014	10.00	0.250	50
3.472	76.028	20.00	0.500	100
6.944	72.556	20.00	0.500	100
13.889	145.111	40.00	1.00	200

EXAMPLE 4

Formulation of Sterile Aqueous Solution of Besylate Salt of Amlodipine

Sodium chloride was dissolved in water for injection and propylene glycol was mixed with this solution. The besylate salt of amlodipine was added and, when it has dissolved, further water for injection was added to adjust the volume to give the desired concentration of amlodipine (1 mg/ml). The solution was then filtered through a sterilising filter and filled into suitable sterile containers, e.g. ampoules, for use in parenteral, e.g. intravenous, administration.

This methods was used to prepare the formulations shown in Table 5.

TABLE 5

STERILE AQUEOUS SOLUTIONS

	(1)	(2)
Besylate salt of amlodipine	1.389 g	1.389 g
Sodium chloride	9.000 g	9.000 g
Propylene glycol	200.000 g	400.000 g
Water for injection	to 1 liter	to 1 liter

EXAMPLE 5

Alternative preparation of Besylate salt of Amlodipine

Ammonium benzenesulphonate (0.943 g) was added to a slurry of amlodipine base (2 g) in industrial methylated spirit (10ml) and the resulting solution was heated at reflux for 10 minutes. The reaction mixture was cooled and granulated at 5° C. for 1 hour. The amlodipine benzenesulphonate was filtered, washed with industrial methylated spirit (2×2 ml) and dried in vacuum.

Yield 1.9 g (70% of theory).

Mpt.: 201.0° C.

Mpt.: 201.0° C. Analysis %	
Found	C, 54.9%; H, 5.46; N, 4.90;
Calculated for	C, 55.07; H, 5.51; N, 4.95.

We claim:

1. The besylate salt of amlodipine.

2. A pharmaceutical composition comprising an anti-hypertensive, antiischaemic or angina - alleviating effective amount of the besylate salt of amlodipine as claimed in claim 1 together with a pharmaceutically acceptable diluent or carrier.

3. A tablet formulation comprising an anti-hypertensive, antiischaemic or angina - alleviating effective amount of the besylate salt of amlodipine as claimed in claim 1 in admixture with excipients.

4. A tablet formulation as claimed in claim 3 wherein the excipients comprise a compression and, an additive to provide sheen to the tablet, a disintegrant and a lubricant.

5. A tablet formulation as claimed in claim 4 wherein the excipients comprise microcrystalline cellulose, anhydrous dibasic calcium phosphate, sodium starch glycollate and magnesium stearate.

6. A capsule formulation comprising an antihypertensive, antiischaemic or angina - alleviating effective amount of the besylate salt of amlodipine as claimed in claim 1 in admixture with excipients.

7. A capsule formulation as claimed in claim 6 wherein the excipients comprise an inert diluent, a dried disintegrant and a lubricant.

8. A capsule formulation as claimed in claim 7 wherein the excipients comprise microcrystalline cellulose, dried maize starch and magnesium stearate.

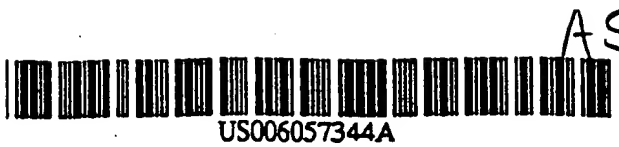
9. A sterile aqueous solution comprising an antihypertensive, antiischaemic or angina - alleviating effective amount of the besylate salt of amlodipine for parenteral administration.

10. A sterile aqueous solution as claimed in claim 9 comprising from 10 to 40% w/v of propylene glycol.

11. A sterile aqueous solution as claimed in claim 9 or claim 10 comprising about 1% w/v sodium chloride.

* * * * *

AST 5



US006057344A

United States Patent [19]

Young

[11] **Patent Number: 6,057,344**

[45] **Date of Patent: May 2, 2000**

[54] **METHODS FOR TREATING HYPERTENSION, AND ANGINA USING OPTICALLY PURE (-) AMLODIPINE**

[75] Inventor: **James W. Young**, Palo Alto, Calif.

[73] Assignee: **Sepracor, Inc.**, Marlborough, Mass.

[21] Appl. No.: **08/334,771**

[22] Filed: **Nov. 4, 1994**

Related U.S. Application Data

[63] Continuation of application No. 07/981,562, Nov. 25, 1992, abandoned, which is a continuation-in-part of application No. 07/798,466, Nov. 26, 1991, abandoned.

[51] Int. Cl.[7] A61K 31/44

[52] U.S. Cl. 514/356

[58] Field of Search 514/356

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,572,909	2/1986	Campbell et al.	514/356
4,806,557	2/1989	Campbell et al.	514/356
4,879,303	11/1989	Davison et al.	514/356

FOREIGN PATENT DOCUMENTS

0 089 167 A2	9/1983	European Pat. Off. .
0 331 315 A2	9/1989	European Pat. Off. .
1-156959	6/1989	Japan .

OTHER PUBLICATIONS

Goldmann, S. et al., "Determination of the Absolute Configuration of the Active Amlodipine Enantiomer as (-)-S: A Correction", *Journal of Medicinal Chemistry* 35(18):3341–3344 (1992).

Alker, D. et al., "Long-acting dihydropyridine calcium antagonists. 9. Structure Activity Relationships Around Amlodipine", *Eur. J. Med. Chem.* 26: 907–913 (1991).

Basco, L.K. and Le Bras, J., "*Plasmodium falciparum*: In Vitro Drug Interaction between Chloroquine and Enantiomers of Amlodipine", *Experimental Parasitology* 72: 262–270 (1991).

Deloron, P. et al., "In Vitro and In Vivo Potentiation of Chloroquine against Malaria Parasites by an Enantiomer of Amlodipine", *Antimicrobial Agents and Chemotherapy* 35(7): 1338–1342 (1991).

Eriksson, U.G. et al., "Stereoselective Metabolism of Felodipine in Liver Microsomes from Rat, Dog, and Human", *Drug Metabolism and Disposition* 19(5): 889–894 (1991).

Goldmann, S. and Stoltefuss, J., "1,4-Dihydroyridines: Effects of Chirality and Conformation on the Calcium Antagonist and Calcium Agonist Activities", *Angew. Chem. Int. Ed. Engl.* 30: 1559–1578 (1991).

Nayler, W.G. and Gu, X.H., "(-)[^{3}H]Amlodipine Binding to Rat Cardiac Membranes", *Journal of Cardiovascular Pharmacology* 17: 587–592 (1991).

Okamoto, Y. et al., "Optical resolution of dihydropyridine enantiomers by high-performance liquid chromatography using phenylcarbamates of polysaccharides as chiral stationary phase", *Journal of Chromatography* 513: 375–378 (1990).

Soons, P.A. et al., "Enantioselective Determination of Felodipine and Other Chiral Dihydropyridine Calcium Entry Blockers in Human Plasma", *Journal of Chromatography* 528: 343–356 (1990).

Abernethy, D.R., "The Pharmacokinetic Profile of Amlodipine", *American Heart Journal* 1100–1103 (1989).

Burges, R.A. et al., "Pharmacologic Profile of Amlodipine", *The American Journal of Cardiology* 64: 10–20 (1989).

Glasser, S.P. et al., "Safety and Efficacy of Amlodipine Added to Hydrochlorothiazide Therapy in Essential Hypertension", *American Journal of Hypertension, Inc.* 2(3)(1): 154–157 (1989).

The Merck Index, Eleventh Edition, Monograph 509 (1989).

Osterloh, I., "The safety of amlodipine", *American Heart Journal* 1114–1120 (1989).

Isenrign, H.P., "Antifungal Morpholine Derivatives: Chemistry and Structure–Activity Relationships", *Recent Trends in the Discovery, Development and Evaluation of Antifungal Agents*, R.A. Fromtling (Ed.), J.R. Prous Science Publishers, S.A., pp. 543–554 (1987).

Arrowsmith, J.E. et al., "Long-Acting Dihydropyridine Calcium Antagonists. 1. 2-Alkoxymethyl Derivatives Incorporating Basic Substituents", *J. Med. Chem.* 29: 1696–1702 (1986).

A.R. Gennaro, Ed., "Remington's Pharmaceutical Sciences, 18th Ed." published by Mack Publishing Co. (Easton, PA), pp. 853–854 (1990).

Ariens, E.J., "Stereoselectivity in Pharmacodynamics and Pharmacokinetics", Schweiz. Med. Wochenschr. 120: 131–134 (1990).

Ariens, E.J., "Racemic Therapeutics—Ethical and Regulatory Aspects", Eur. J. Clin. Pharmacol. 41:89–93 (1991).

J. Luksa et al., "Pharmacokinetic Behaviour of R-(+)-and S-(-)-Amlodipine After Single Enantiomer Administration," *Journal of Chromatography B*, 703:185–193 (1997).

H. Laufen et al., "Enantioselective Disposition of Oral Amlodipine in Healthy Volunteers," *Chirality*, 6:531–536 (1994).

Primary Examiner—William R. A. Jarvis

Attorney, Agent, or Firm—Pennie & Edmonds LLP

[57] **ABSTRACT**

Methods are disclosed utilizing the optically pure (-) isomer of amlodipine. This compound is a potent drug for the treatment of hypertension while avoiding the concomitant liability of adverse effects associated with the racemic mixture of amlodipine. The (-) isomer of amlodipine is also useful for the treatment f angina without the concomitant liability of adverse effects associated with the racemic mixture of amlodipine.

16 Claims, No Drawings

METHODS FOR TREATING HYPERTENSION, AND ANGINA USING OPTICALLY PURE (-) AMLODIPINE

This is a continuation of application Ser. No. 07/981,562 filed Nov. 25, 1992, now abandoned, which is a continuation-in-part of application Ser. No. 07/798,466 filed Nov. 26, 1991, now abandoned, each of which is incorporated by reference herein in its entirety.

BACKGROUND OF THE INVENTION

This invention relates to novel compositions of matter containing optically pure (-) amlodipine. These compositions possess potent activity in treating both systolic and diastolic hypertension while avoiding adverse effects including but not limited to edema of the extremities, headache and dizziness, which are associated with administration of the racemic mixture of amlodipine. Additionally, these novel compositions of matter containing optically pure (-) amlodipine are useful in treating angina and such other conditions as may be related to the activity of (-) amlodipine as a calcium channel antagonist including but not limited to cerebral ischemia, cerebral disorders, arrhythmias, cardiac hypertrophy, coronary vasospasm, myocardial infarction, renal impairment and acute renal failure—while avoiding the adverse effects associated with administration of the racemic mixture of amlodipine. Also disclosed are methods for treating the above-described conditions in a human while avoiding the adverse effects that are associated with the racemic mixture of amlodipine, by administering the (-) isomer of amlodipine to said human.

Steric Relationship and Drug Action

Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric or racemic mixture.

Stereochemical purity is of importance in the field of pharmaceuticals, where 12 of the 20 most prescribed drugs exhibit chirality. A case in point is provided by the L-form of the β-adrenergic blocking agent, propranolol, which is known to be 100 times more potent than the D-enantiomer.

Furthermore, optical purity is important since certain isomers may actually be deleterious rather than simply inert. For example, it has been suggested that the D-enantiomer of thalidomide was a safe and effective sedative when prescribed for the control of morning sickness during pregnancy, while the corresponding L-enantiomer has been thought to be a potent teratogen.

The active compound of this composition and method is an optical isomer of the compound amlodipine, which is described in Davison et al., U.S. Pat. No. 4,572,909. Chemically, this (-) isomer is 3-ethyl 5-methyl(-)-2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine-dicarboxylate. This isomer will hereinafter be referred to as (-) amlodipine. (-) Amlodipine also includes the substantially optically pure (-) amlodipine isomer.

Amlodipine, which is the subject of the present invention, is available commercially only as the 1:1 racemic mixture. That is, it is available only as the 1:1 mixture of optical isomers, called enantiomers. The racemic mixture of amlodipine that is commercially available for administration is a besylate salt.

Pharmacologic Action

The racemic mixture of amlodipine is in the class of compounds known as calcium antagonists. The concept of a specific mechanism of pharmacologic action related to the antagonism of calcium movement in the process of excitation-contraction was suggested by Fleckenstein et. al. (see Fleckenstein, A., *Calcium Antagonism in Heart and Smooth Muscle: Experimental Facts and Therapeutic Prospects*, New York, Wiley, 1983; Swamy, V. and D. Triggle, *Modern Pharmacology*, 2nd. Ed., Craig and Stitzel, Eds., Little, Brown and Co., Boston, 1986, Chapt. 26, 373–380; Triggle, D. J., and R. A. Janis, *Ann. Rev. Pharm. and Tox.* 27: 347–369, 1987). Many of the currently available calcium antagonists appear to antagonize the entry of calcium through voltage dependent channels in the plasma membrane of cells. The pharmacologic class of calcium antagonists consists of chemically diverse compounds. Given the structural heterogeneity of the class it is likely that the pharmacological action involves more than one site or mechanism of action.

Amlodipine is one of a series of dihydropyridine calcium antagonists. However, amlodipine has a generally slower onset and longer duration of action than, for example, nifedipine. (Jensen, H. et al., *J. Hum. Hypertens.*, 42(5): 541–45, 1990). The metabolites of amlodipine apparently do not possess significant calcium channel blocking activity, while the parent drug offers a biological half-life of some 35–40 hours, prompting a once-daily dosage regimen. (Lorimer, A. R., et al., *J. Hum. Hypertens.*, 3(3): 191–96, 1989; Glasser, S. F. et al., *AJH*, 2(3): 154–57, 1989). Its ability to block calcium channels in smooth muscle produces peripheral vasodilation resulting in decreases in both systolic and diastolic blood pressure.

With regard to the enantiomers of amlodipine, these are disclosed in Arrowsmith et al. *J. Med. Chem.*, 29: 1696–1702 (1986). This reference discusses in vitro tests to determine calcium antagonist activity against calcium-induced constriction of potassium-depolarized rat aorta. The authors allege that in vitro evaluation of the enantiomers of amlodipine shows the (-) isomer to be twice as active as the racemic mixture in antagonizing calcium-induced constriction of potassium-depolarized rat aorta. The authors also allege that the (+) isomer of amlodipine is some 1,000 times less active in these in vitro tests. Further, European Patent Application No. 0 331 315 discloses a method for separation of the R(-) and S(+) isomers of amlodipine. While these references discuss the enantiomers of amlodipine, they only provide a method of synthesis and in vitro test results of the individual enantiomer activity as found in rat aorta. There is no disclosure of the effects of or a suggestion for administering one of the enantiomers of amlodipine to a human. Moreover, there is no disclosure or suggestion of the alleviation or reduction of side effects which is achieved by the administration of (-) amlodipine.

The racemic mixture of amlodipine is presently used primarily as an antihypertensive agent; it is generally taken orally as a once-daily therapy. As stated above, the racemic mixture of amlodipine produces peripheral vasodilation, resulting in decreases in both systolic and diastolic blood

pressure when used as an antihypertensive agent. This antihypertensive effect occurs in the relative absence of significant or sustained effects on cardiac rate.

While yet the subject of extensive research, hypertension appears to be the product of an inherited predisposition—coupled with dietary, emotional, and environmental factors, which results in a structural adaptation of the cardiac muscle and the large blood vessels. Most patients display heightened vascular and cardiac reactions to sympathetic nervous stimulation, but the precise relationship of sympathetic nervous stimulation to the etiology of the disease. Nevertheless, hypertension results in chronic readjustment of cardiovascular hemodynamics, alteration of blood vessel walls, cardiovascular resistance and regional transmural pressures.

Pharmacologic management of hypertension is generally directed to the normalization of altered hemodynamic parameters, and many drugs and drug classes, either as monotherapy or in combination treatment, can reduce and control elevated blood pressure. However, treatment of hypertension does not always correspondingly benefit the morbidity and mortality of the condition, either because chronic hypertension has produced other significant and irreversible cardiovascular changes, or because present drugs have an adverse effect on some other risk factor for cardiovascular disease. Rather, current drug therapy simply provides sustained arterial pressure reduction.

Furthermore, the racemic mixture of amlodipine is useful in treating other disorders such as angina pectoris.

Angina pectoris is a highly variable, rather poorly understood clinical syndrome reflecting a myocardial ischemia. When cardiac work or myocardial oxygen demand exceeds the ability of the coronary arterial vascular system to supply oxygen, the resulting ischemia stimulates the sensory nerves of the heart, producing the sensation of angina characterized by episodes of precordial pressure, discomfort, or a severe, intense crushing pain which may radiate to several sites including the left shoulder and left arm. Physical activity or exertion characteristically initiates the condition, and rest or drug therapy relieves the condition. The signs and symptoms of an episode persist for a few minutes, but can be induced or exaggerated by a meal or exposure to cold air. Treatment is directed to the underlying disease, usually atherosclerosis, or to drugs which either reduce myocardial oxygen demand or improve oxygen supply. Calcium antagonists such as amlodipine have been particularly useful in treating vasospastic angina, the angina of effort, and the unstable angina, due to the effect of the calcium channel antagonist on cardiac and vascular smooth muscle.

Amlodipine may be useful in the treatment of cerebral ischemia. Cerebral ischemia, often the result of atherosclerotic disease or hypertension, results from insufficient cerebral circulation. Under normal circumstances, an extensive collateral circulation ensures adequate blood flow. However, cerebral ischemia may result from either an intra- or extracranial interruption of arterial blood flow. If interruption is transient, the cerebral tissues recover, and neurologic symptoms disappear. If the ischemia lasts for a somewhat more extended period, infarction results and the resulting neurologic damage is permanent. In the case of extended ischemia resulting in infarction, treatment is directed to the underlying vascular disease, to blood platelet aggregation inhibitors, and anticoagulant therapy.

Because of its activity as a calcium channel antagonist, amlodipine may also be useful in treating cardiac arrhythmias. Cardiac arrhythmias represent a broad, complex group of electrophysiologic disorders that affect the mechanical properties f the heart and vasculature, altering normal cardiac rhythm, function and output. Normal cardiac rhythm originates with the sinoatrial node, which possesses high intrinsic automaticity. Adequate automaticity and conduction lead to activation of atrial and ventricular fibers, producing in sequence the elements of normal functional heart beat. Calcium antagonists may be of value in conditions where calcium-related changes in membrane potential and conduction alter normal rhythm. In the absence of treatment, symptoms vary with individual arrhythmias, but are often the consequence of inadequate cardiac filling and output and often include fatigue, decreased exercise tolerance, syncope, shortness of breath, nausea, lightheadedness and the like.

Amlodipine may be useful to treat cardiac hypertrophy. Cardiac hypertrophy can result from excessive workload either due to an obstruction to outflow, termed systolic overload, or to excessive volumes presented to the heart in diastole, termed diastolic overload. Systolic overload results in concentric ventricular hypertrophy, in which there is an increased thickness in the walls of the heart not associated with increased volume. Diastolic overload causes dilation and hypertrophy with an increased blood volume. An inadequate cardiac output results from the heart's failure in systolic or diastolic overload, leading to fatigue, shortness of breath, pulmonary congestion, edema and the like. Calcium channel antagonists effect workload and, as such, may be useful in treating cardiac hypertrophy due to the effect of the calcium antagonist on cardiac and vascular smooth muscle in reducing blood pressure.

It is also possible that amlodipine could be used to treat coronary arterial spasm. Coronary arterial spasm can occur in the absence of significant coronary atherosclerosis and is thought to be an initiating event in variant angina and in myocardial infarction. Coronary spasm may occur without the patient feeling any significant discomfort. In an electrically unstable heart, diverse neural impulses to the heart may provoke coronary vascular spasm. This may result in enhanced myocardial ischemia and arrhythmia, which in turn may culminate in ventricular fibrillation and sudden cardiac death. As in variant or vasospastic angina, the calcium channel antagonists may be of particular usefulness due to their effect on cardiac and vascular smooth muscle.

Furthermore, amlodipine may be useful in the treatment of myocardial infarction, ischemic myocardial necrosis, and ischemia reperfusion injury. Myocardial infarction or ischemic myocardial necrosis generally results from the abrupt reduction of coronary blood flow to a portion of the myocardium. The condition likely originates from atherosclerosis of the coronary arteries. Either coronary artery vasospasm or acute coronary thrombosis precipitates the condition, although the etiology is the subject of continuing research. Myocardial infarction is predominantly a disease of the left ventricle. Precordial pain and left ventricular dysfunction characterize the disease. The pain, which can be severe aching or pressure, leads to apprehension. Symptoms include left ventricular heart failure, pulmonary edema, shock or significant cardiac arrhythmia. Calcium channel antagonists may find utility in the management of myocardial infarction patients due to their effects on coronary artery vasospasm, blood pressure or other effects on cardiac function or vascular smooth muscle.

Amlodipine may be used to treat congestive heart failure. Congestive heart failure can be caused by hypertension, cardiomyopathy, coronary artery disease or valvular heart disease. Congestive failure results in poor cardiac output and elevated left-ventricular diastolic pressure, leading to

dyspnea, fatigue, peripheral edema, and coughing. The ability of some calcium antagonists to lower afterload by dilating peripheral arteries without having a significant inotropic effect may increase their use in treating congestive heart failure.

Amlodipine may be of use in treating migraine. Classic migraine typically begins with visual auras followed by severe headaches, often accompanied by nausea and vomiting. Common migraine has similar symptoms without the preceding visual aura. The causes of migraine have been studied intensely, and are still a matter of debate. The most generally accepted cause is hypoxia due to reduced cerebral blood flow. Calcium channel antagonists have been used for migraine prophylaxis since they can increase cerebral blood flow.

Amlodipine may also be useful for treating Raynaud's phenomenon, which is characterized by vascular spasm of the extremities. These vasospasms can be caused by cold or stress. A pallor or cyanosis is usually present due to severe constriction of the digital arteries. The phenomenon is often seen as a secondary disorder with arterial diseases or connective tissue diseases such as scleroderma, arthritis or lupus erythematosus. Calcium channel antagonists have been shown to be effective in treating Raynaud's phenomenon.

Amlodipine may be useful in the treatment of asthma and bronchospasm. Symptoms of asthma—coughing, wheezing, and dyspnea—are caused by constriction of tracheobronchial smooth muscle. Asthma attacks can be triggered by antigenic stimuli (pollen, dust) or non-antigenic stimuli (exercise, pollution, infection). The response to these stimuli lead to secretions of chemical mediators that cause smooth muscle contraction. Calcium channel antagonists can be used to control bronchoconstriction and relieve asthma attacks.

In addition, the racemic mixture of amlodipine may be useful to treat renal impairment and acute renal failure. Renal impairment and acute renal failure are clinical conditions of diverse etiology, which are associated with an increasing azotemia or urea nitrogen in the blood, and often an oliguria or a diminished volume of urine in relation to fluid intake. The pathophysiology may originate prerenally, manifest as inadequate renal perfusion, due to extracellular fluid volume depletion or cardiac failure. The most common cause of intrinsic renal failure is prolonged renal ischemia. Postrenal azotemia may be associated with obstruction or renal glomerular and tubular dysfunction. Laboratory findings disclose progressive azotemia, acidosis, hyperkalemia, and hyponatremia. Factors aggravating kidney impairment or failure must be specifically treated, including heart failure, obstruction and the like. Moderate or severe hypertension has a deleterious effect on renal function, and management of the hypertension with a variety of drugs including calcium channel antagonists may be useful therapy.

In addition, the racemic mixture of amlodipine could be useful in the treatment of cognitive disorders. Cognitive disorders include but are not limited to dementia and age-associated memory impairment.

Dementia can occur at any age. It is a structurally caused permanent or progressive decline in several dimensions of intellectual function that interferes substantially with individual normal social or economic activity.

One particular type of dementia is Alzheimers-type dementia. Alzheimers-type of dementia is thought to be due to a degenerative process, with a large loss f cells from the cerebral cortex and other brain areas. Acetylcholine-transmitting neurons and their target nerve cells are particularly affected. The brain shows marked atrophy with wide sulci and dilated ventricles. Senile plaques and neurofibrillary tangles are present. Memory loss is the most prominent early symptom. Disturbances of arousal do not occur early in the course. Alzheimer's presenile and senile onset dementias are similar in both clinical and pathologic features, with the former commonly beginning in the 5th and 6th decades and the latter in the 7th and 8th decades. The dementia usually progresses steadily, becoming well advanced in 2 to 3 years. Some cases of dementia occurring in the presenile period are hard to classify and are sometimes labelled idiopathic or simple presenile dementia.

The signs and symptoms of dementia in particular Alzheimers-type dementia include depression, paranoia, anxiety or any of several other psychologic symptoms. The most common clinical picture is slow disintegration of personality and intellect due to impaired insight and judgment and loss of affect. Memory impairment increases, beginning with problems recalling recent events or finding names. The impairment varies greatly from time to time and often from moment to moment. Dementia generally is an insidious, slowly progressive, untreatable condition. However, the rate of progression varies widely and depends on the cause.

Another type of cognitive disorder is age-associated memory impairment (AAMI). AAMI is used to describe healthy non-demented people who have experienced memory loss over the course of the person's life. Most commonly it is used to describe adults over the age of 50 who have experienced memory loss over the course of adult life. It has been estimated that between 25% and 50% of people over the age of 65 have this disorder.

Many calcium channel antagonists cause significant adverse effects. These adverse effects include but are not limited to tachycardia, orthostatic hypotension and fluid retention. In contrast to the situation with several other calcium channel antagonists, however, the racemic mixture of amlodipine has not been found to cause either marked or prolonged direct effects on heart rate or the reflex consequence of vasodilation.

However, the administration of the racemic mixture of amlodipine to a human has been found to cause still other adverse effects. These adverse effects include but are not limited to edema of the extremities including peripheral edema, headache, flushing/hot flashes, fatigue, vertigo, muscle cramps and dizziness.

Thus, it would be particularly desirable to find a compound with the advantages of the racemic mixture of amlodipine which would not have the aforementioned disadvantages of significant adverse side effects and which was useful for treatment of other conditions.

SUMMARY OF THE INVENTION

It has now been discovered that the optically pure (-) isomer of amlodipine is an effective antihypertensive agent for both systolic and diastolic hypertension, particularly in mild to moderate disease and angina, while avoiding adverse effects including but not limited to edema f the extremities, headache and dizziness, which are associated with the administration of the racemic mixture of amlodipine. It has also been discovered that these novel compositions of matter containing optically pure (-) amlodipine are useful in treating ther conditions as may be related to the activity f (-) amlodipine as a calcium channel antagonist, including but not limited to cerebral ischemia, cerebral disorders,

arrhythmias, cardiac hypertrophy, coronary vasospasm, myocardial infarction, renal impairment and acute renal failure while avoiding the above-described adverse effects associated with the administration of the racemic mixture f amlodipine. The present invention also includes methods for treating the above-described conditions in a human while avoiding the adverse effects that are associated with the racemic mixture of amlodipine by administering the (-) isomer of amlodipine to said human.

DETAILED DESCRIPTION OF THE INVENTION

The present invention encompasses a method of eliciting an antihypertensive effect in a human, while avoiding the concomitant liability of adverse effects, which comprises administering to said human in need of such antihypertensive therapy, an amount of (-) amlodipine or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate hypertension, but insufficient to cause said adverse effects associated with administration of racemic amlodipine.

The present invention also encompasses an antihypertensive composition for the treatment of a human in need of antihypertensive therapy, which comprises an amount of (-) amlodipine or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate said hypertension but insufficient to cause adverse effects of racemic amlodipine.

The present invention further encompasses a method of treating angina in a human, while avoiding the concomitant liability of adverse effects, which comprises administering to said human in need of such anti-angina therapy, an amount of (-) amlodipine, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate said condition but insufficient to cause said adverse effects associated with administration of racemic amlodipine.

In addition, the present invention encompasses an antianginal composition for the treatment of a human having angina, which comprises an amount of (-) amlodipine or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate said angina but insufficient to cause adverse effects of racemic amlodipine.

A further aspect of the present invention includes a method of treating a condition caused by excessive calcium influx in cells in a human, while avoiding the concomitant liability of adverse effects, which comprises administering to said human in need of a reduction in excessive calcium influx an amount of (-) amlodipine, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, sufficient to alleviate said condition but insufficient to cause said adverse effects of racemic amlodipine. Conditions caused by excessive calcium influx in cells in a human include but are not limited to cerebral ischemia, cerebral disorders such as cognitive disorders including but not limited to Alzheimer's dementia and memory impairment, arrhythmias, cardiac hypertrophy, congestive heart failure, coronary vasospasm, migraine, bronchospasm and asthma, Raynaud's phenomenon, myocardial infarction, renal impairment and acute renal failure.

Furthermore, the present invention includes a composition for treating a condition caused by excessive calcium influx in cells in a human, which comprises an amount of (-) amlodipine, r a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate said condition but insufficient to cause adverse effects f racemic amlodipine.

The commercially available racemic mixture of amlodipine (e.g., a 1:1 racemic mixture of the two enantiomers) causes antihypertensive and antianginal activity; however, this racemic mixture, while offering the expectation of efficacy, causes adverse effects. Utilizing the (-) isomer of amlodipine results in clearer dose-related definitions of efficacy, surprisingly diminished adverse effects, and accordingly, an improved therapeutic index. It is, therefore, more desirable to use the (-) isomer of amlodipine.

The term "adverse effects" includes, but is not limited to, cardiovascular effects (including tachycardia and diminished contractility of the heart), edema of the extremities, headache, dizziness, flushing, fatigue, vertigo, and muscle cramps.

The term "substantially free of its (+) stereoisomer" as used herein means that the composition contains a greater proportion or percentage of the (-) isomer of amlodipine in relation to the (+) isomer of amlodipine, said percentage being based on the total amount of amlodipine. In a preferred embodiment the term "substantially free of its (+) stereoisomer means that the composition contains at least 90% by weight of (-) amlodipine, and 10% by weight or less of (+) amlodipine. In the most preferred embodiment the term "substantially free of the (+) stereoisomer" means that the composition contains at least 99% by weight (-) amlodipine, and 1% or less of (+) amlodipine. In another preferred embodiment the term "substantially free of its (+) stereoisomer" as used herein means that the composition contains 100% by weight of (-) amlodipine. The terms "substantially optically pure (-) isomer of amlodipine" and "optically pure (-) isomer of amlodipine are also encompassed by the above-described meanings.

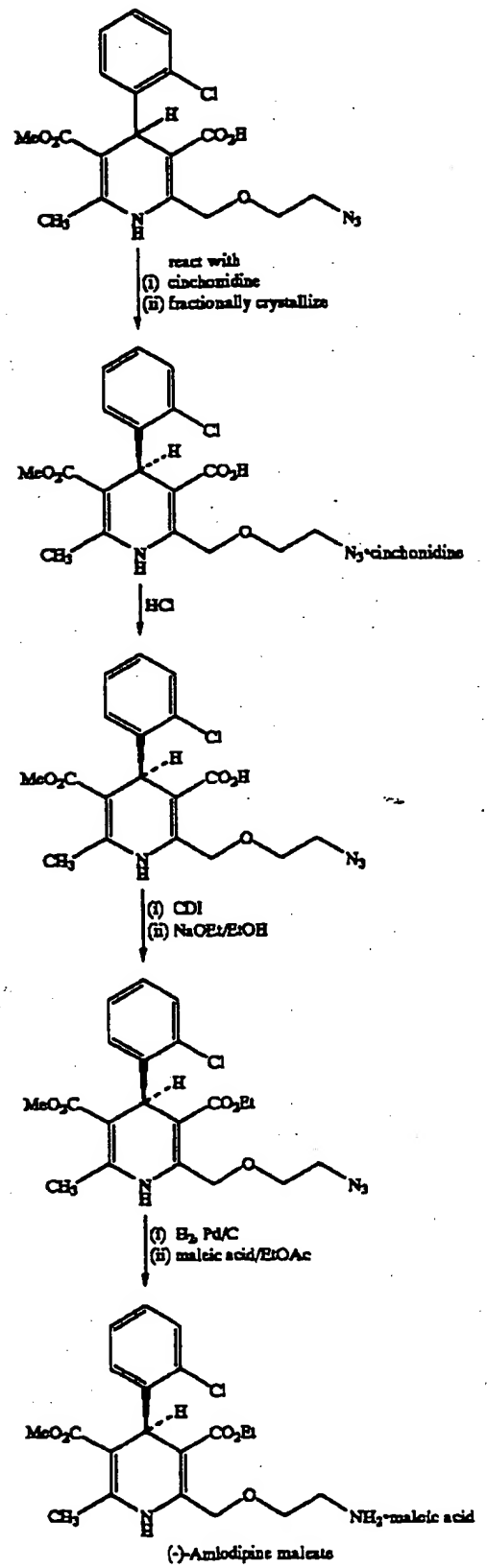
The term "eliciting an antihypertensive effect" as used herein means providing a normalization to otherwise elevated systolic and/or diastolic blood pressure, and by so doing providing relief from any possible symptoms or other hemodynamic effects caused by the elevated pressure.

The term "a method of treating angina" as used herein means relief from the symptoms of myocardial ischemia, which include, but are not limited to, episodes of precordial pressure, discomfort, or a severe intense, crushing pain which may radiate, and which may be accompanied by changes in respiration, pulse rate, and blood pressure.

The term, "a condition caused by excessive calcium influx in cells in a human" includes but is not limited to conditions involving calcium influx in human cell that may be present in smooth muscle, cardiac, and other tissues including lung and brain. These conditions include, but are not limited to, cerebral ischemia, cerebral disorders such as cognitive disorders including Alzheimer's dementia and memory impairment, arrhythmias, cardiac hypertrophy, congestive heart failure, coronary vasospasm, migraine, bronchospasm and asthma, Raynaud's phenomenon, myocardial infarction, renal impairment and acute renal failure. The symptoms associated with these disorders include, but are not limited to, the symptoms of precordial discomfort or pain, headache, fatigue, decreased exercise tolerance, syncope, shortness of breath, nausea, lightheadedness, edema, pulmonary congestion, arrhythmia or palpitation, azotemia, and/or oliguria.

The chemical synthesis of the racemic mixture of amlodipine can be performed by the method described in Arrowsmith, J. E. et al., *J. Med. Chem.*, 29: 1696–1702 (1986).

A technique for separation of the (−) amlodipine isomer from the racemic mixture is illustrated schematically (see Arrowsmith, J. E., EP 331,315) as follows:



The racemic acid 1 is converted to its cinchonidine salts in methanol solution. Upon dilution with water and standing at room temperature, a crystalline precipitate is formed which can be subsequently recrystallized to constant rotation to give the diastereomerically pure cinchonidine salt 2. Further, the mother liquids from the original crystallization can be reduced in volume and stirred at room temperature, e.g. overnight, to afford a fine precipitate which can also be recrystallized to give the diastereomerically pure cinchonidine salt 2. The cinchonidine salt 2 is partitioned between ethyl acetate and dilute hydrochloric acid to liberate the (+) acid 3. The acid 3 is then esterified using carbonyldimidazole (CDI) in near-quantitative yield by forming an imidazolide and decomposing the imidazolide with ethanolic sodium ethoxide to give 4. The azido group in 4 can then be cleanly reduced to amino by catalytic hydrogenation, giving (−) amlodipine, which is most conveniently isolated as the salt of an acid, e.g. as the maleate 5.

The magnitude of a prophylactic or therapeutic dose of (−) amlodipine in the acute or chronic management of disease will vary with the severity of the condition to be treated and the route of administration. The dose, and perhaps the dose frequency, will also vary according to the age, body weight, and response of the individual patient. In general, the total daily dose ranges, for the conditions described herein, is from about 0.01 mg. to about 100.0 mg. Preferably, a daily dose range should be between about 0.5 mg to about 20.0 mg. while most preferably, a daily dose range should be between about 0.5 mg to about 10 mg. In managing the patient, the therapy should be initiated at a lower dose, perhaps about 0.025 mg to about 2.5 mg and increased up to about 20 mg or higher depending on the patient's global response. It is further recommended that children and patients over 65 years, and those with impaired renal or hepatic function, initially receive low doses, and that they be titrated based on global response and blood level. It may be necessary to use dosages outside these ranges in some cases.

The various terms, "an amount sufficient to alleviate hypertension but insufficient to cause said adverse effects, "an amount sufficient to alleviate said condition but insufficient to cause said adverse effects" wherein said condition is angina; and "an amount sufficient to alleviate said condition but insufficient to cause said adverse effects" wherein said condition includes but is not limited to cerebral ischemia, cerebral disorders, arrhythmias, cardiac hypertrophy, coronary vasospasm, myocardial infarction, renal impairment and acute renal failure are encompassed by the above described dosage amounts and dose frequency schedule.

Any suitable route of administration may be employed for providing the patient with an effective dosage of (−) amlodipine. For example, oral, rectal, parenteral, transdermal, subcutaneous, intramuscular, and the like may be employed. Dosage forms include tablets, troches, dispersions, suspensions, solutions, capsules, patches, and the like.

The pharmaceutical compositions of the present invention comprise (−) amlodipine as active ingredient, r a pharmaceutically acceptable salt thereof, and may also contain a pharmaceutically acceptable carrier, and optionally, other therapeutic ingredients.

The term "pharmaceutically acceptable salts" refers to salts prepared from pharmaceutically acceptable non-toxic acids including inorganic acids and organic acids.

Since the compound of the present invention is basic, salts may be prepared from pharmaceutically acceptabl non-toxic acids including inorganic and organic acids. Such acids

include acetic, benzene-sulf nic (besylate), benzoic, camphorsulfonic, citric, ethenesulfonic, fumaric, gluconic, glutamic, hydrobromic, hydrochloric, isethionic, lactic, maleic, malic, mandelic, methanesulfonic, mucic, nitric, pamoic, pantothenic, phosphoric, succinic, sulfuric, tartaric acid, p-toluenesulfonic, and the like. Particularly preferred are besylate, hydrobromic, hydrochloric, phosphoric and sulfuric acids. (See Campbell, S. F. et al., U.S. Pat. No. 4,806,557.)

The compositions include compositions suitable for oral, rectal and parenteral (including subcutaneous, intramuscular, and intravenous) administration, although the most suitable route in any given case will depend on the nature and severity of the condition being treated. The most preferred route of the present invention is the oral route. The compositions may be conveniently presented in unit dosage form, and prepared by any of the methods well known in the art of pharmacy.

In the case where an oral composition is employed, a suitable dosage range for use is from about 0.01 mg. to about 100.0 mg. total daily dose, given as a once daily administration in the morning or in divided doses if required. Preferably, a dose range of between about 0.5 mg to about 20.0 mg is given as a once daily administration or in divided doses if required, and most preferably a dose range of from between about 0.5 mg to about 10.0 mg is given as a once daily administration or in divided doses if required. Patients may be upward titrated from below to within this dose range to a satisfactory control of symptoms or blood pressure as appropriate.

In practical use, (−) amlodipine can be combined as the active ingredient in intimate admixture with a pharmaceutical carrier according to conventional pharmaceutical compounding techniques. The carrier may take a wide variety of forms depending on the form of the preparation desired for administration, e.g., oral or parenteral (including intravenous injections or infusions). In preparing the compositions for oral dosage form any of the usual pharmaceutical media may be employed. Usual pharmaceutical media include, for example, water, glycols, oils, alcohols, flavoring agents, preservatives, coloring agents, and the like in the case of oral liquid preparations (such as for example, suspensions, solutions, and elixirs); aerosols; or carriers such as starches, sugars, microcrystalline cellulose, diluents, granulating agents, lubricants, binders, disintegrating agents and the like, in the case of oral solid preparations (such as for example, powders, capsules, and tablets) with the oral solid preparations being preferred over the oral liquid preparations. The most preferred oral solid preparation is tablets.

Because of their ease of administration, tablets and capsules represent the most advantageous oral dosage unit form, in which case solid pharmaceutical carriers are employed. If desired, tablets may be coated by standard aqueous or nonaqueous techniques.

In addition to the common dosage forms set out above, the compounds of the present invention may also be administered by controlled release means and/or delivery devices such as those described in U.S. Pat. Nos.: 3,845,770; 3,916,899; 3,536,809; 3,598,123; and 4,008,719, the disclosures of which are hereby incorporated by reference.

Pharmaceutical compositions f the present inventi n suitable for oral administration may be presented as discrete units such as capsules, cachets, or tablets, r aerosols sprays, each containing a predetermined amount of the active ingredient, as a powder or granules, or as a solution or a suspension in an aqueous liquid, a non-aqueous liquid, an il-in-water emulsion, or a water-in-oil liquid emulsion. Such compositions may be prepared by any of the methods f pharmacy, but all methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more necessary ingredients. In general, the compositions are prepared by uniformly and intimately admixing the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired presentation.

For example, a tablet may be prepared by compression or molding, optionally, with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as powder or granules, optionally mixed with a binder, lubricant, inert diluent, and/or surface active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. Desirably, each tablet contains from about 0.01 mg to about 50 mg of the active ingredient, and each cachet or capsule contains from about 0.5 mg to about 50 mg of the active ingredient, (−) amlodipine. Most preferably, the tablet, cachet or capsule contains either one of three dosages, 0.5 mg, 2.5 mg and 5.0 mg (as scored tablets, the preferable dose form) of the active ingredient.

The invention is further defined by reference to the following examples describing in detail the testing and preparation of the compositions of the present invention. It will be apparent to those skilled in the art, that many modifications, both to materials and methods, may be practiced without departing from the purpose and interest of this invention.

EXAMPLES

Example 1

Vascular Selectivity Studies

The relative potency of optically pure (−) amlodipine and racemic amlodipine as calcium channel antagonists and negative inotropic agents are determined by a pharmacological study. Evaluation of these compounds and others in in vitro test systems provide results, from which the vascular selectivity of a particular compound can be assessed. Calcium channel antagonist activity of the compounds as a function of their molar concentration can be evaluated by measuring their inhibition of the calcium-induced contraction of strips of rat aorta immersed in a bath of Krebs-Henseleit buffer containing 45 mM K+and no Ca^{2+}. In the presence of various concentrations of the antagonists, inhibition would occur in the contraction of this isolated tissue preparation in response to the addition of calcium chloride. Antagonists may be compared by examining the molar concentration of compounds inhibiting the calcium-induced contraction by 50%.

As an index of cardiac depression, negative inotropic activity may be comparably assessed using isolated heart preparations of adult rats. The tissues are prepared and perfused in vitro with Krebs-Henseleit buffer solution, with the activity of the calcium channel antagonists evaluated as a function of their concentration. The compounds are tested for their ability to alter cardiac contraction. Relative potency is calculated from the IC_{25} values of the compounds, i.e., the concentration required to depress contraction by 25%.

Example 2

Radioligand Binding Studies

Hind limb skeletal muscles from rats r guinea pigs are minced and homogenized. After filtration and repeated

centrifugation, the pellet is homogenized and diluted in a Tris buffer to a protein concentration of 1–3 mg/ml. Volumes of this suspension containing 3–10 μg protein are incubated in the presence of a fixed concentration of 0.2 to 0.5 nM (+)-[^{3}H]-isradipine or a similar radioactive ligand and increasing concentrations of racemic amlodipine, (–) amlodipine or (+) amlodipine. After 1 hour incubation, the bound and free radioactivity is measured in a scintillation counter and the affinity of the test compounds to the receptors is calculated.

Example 3

Effects on Coronary Vascular Resistance in the Guinea Pig Langendorff Heart Preparation

Male guinea pigs weighing between 400 and 450 g are killed by cervical dislocation. The hearts are removed and perfused with Krebs-Henseleit solution at constant pressure (60 cm water) by means of retrograde cannulation of the aorta in a Langendorff apparatus. The Krebs-Henseleit solution, consisting of 118.0 mM NaCl, 4.7 mM KCl, 5.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 25.0 mM $NaHCO_3$ and 5.0 mM glucose, is prewarmed to 37° C. and gassed with a mixture of 95% oxygen/5% carbon dioxide. A balloon catheter connected to a pressure transducer is placed in the left ventricle via the left atrium and is preloaded to a pressure of 40 mm Hg. Coronary perfusate flow is measured continuously, and changes in heart rate and left ventricular contractility are also monitored continuously.

Each experiment consists of a 30 minute equilibrium period during which coronary flow is stabilized at 9–12 ml/min. Following this period, a vasoconstrictor is injected 3 times at 40 minute intervals into the cannulated aorta. This dose of U-46619 (9,11-methanoepoxy-PGH_2) evokes approximately a 75% decrease in coronary flow within 30–40 sec, and the effect is fully reversible after 20–25 min continuous perfusion. Racemic amlodipine, (–) amlodipine or (+) amlodipine dissolved in dimethyl sulfoxide or the vehicle are injected in increasing concentrations prior to further U-46619 injections.

The mean decrease in coronary flow obtained with three consecutive injections of U-46619 in the absence of the test substance is taken to be 100% and the percent inhibition of this effect in the presence of increasing concentrations of the test drugs is calculated. Complete individual dose-response curves for each test drug are generated in five hearts, enabling the calculation of the dose for the half-maximal antivasoconstrictor effect (ID_{50}).

Example 4

Antihypertensive Efficacy in Spontaneously Hypertensive Rats

Male spontaneously hypertensive rats (300–350 g) are anesthetized, and polyethylene catheters are implanted in the abdominal aorta via a femoral artery, and in the abdominal vena cava via a femoral vein. The arterial catheters are connected to pressure transducers by means of an intraflow device, flushing the catheters with 3 ml/hr. Mean arterial pressures are derived electronically from the blood pressure wave. Mean pretreatment values of mean arterial pressure are in the range of 160–220 mm Hg. Doses of racemic amlodipine, (–) amlodipine and (+) amlodipine, or of the solvent vehicle, are injected into the venous catheter. Responses in mean arterial pressure to the respective drug or solvent are registered and the relative potencies of the test compounds are calculated.

Example 5

Cardiovascular

Calcium Antagonism, Guinea Pig Ileum (in vitro)

Test substance (3 μg/ml) inhibition of the contractile response of the K^+-depolarized isolated guinea pig ileal segment, bathed in Ca^{++}-free physiological salt solution at 37° C., to added calcium (20 μg/ml of $CaCl_2$), indicates calcium antagonist activity.

Reference Agents (ED_{100}, μg/ml)

atropine	>2	isoxuprine	4
cinnarizine	1	mepyramine	>5
cyproheptadine	0.025	nifedipine	0.001
diltiazem	0.01	papaverine	4
diphenhydramine	1	promethazine	0.25
flunarizine	0.1	propranolol	4
ipratropium bromide	>2	verapamil	0.01

Example 6

Studies on Insulin Resistance

Insulin is a hormone that activates various biochemical processes in the body, the most well known being facilitation of glucose transport over cell membranes and activation of cell growth. The development of insulin resistance is common both in diabetics and nondiabetics, but it is only the glucose transport system that develops resistance to insulin. To compensate for the impaired glucose transport, the normal body produces more insulin and the diabetic patient has to inject higher doses of insulin. Since insulin also is a growth hormone, the increased insulin concentration induces an accelerated growth of atherosclerotic lesions and increased risk for cardiovascular morbidity and mortality.

The present studies are performed in old, spontaneously hypertensive rats (SHRs), which are known to develop insulin resistance. Racemic amlodipine, (–) amlodipine, and (+) amlodipine are studied for their effects on glucose transport, insulin plasma concentration and arterial blood pressure.

Prior to receiving vehicle or test compound, basal measurements of the following parameters are made: (1) systolic blood pressure (measured via tail cuff occlusion); (2) fasting levels of plasma insulin and triglycerides; and (3) glucose tolerance.

The SHRs receive vehicle or test compound via oral gavage once or twice daily for two or four weeks. Measurements of blood pressure, circulating insulin and triglycerides, and glucose clearance are made following two (and four) weeks of drug administration. Any changes in insulin resistance resulting from the drug treatment are evident as changes in the ratio of plasma glucose/plasma insulin levels and from the glucose tolerance tests.

Example 7

Oral F rmulation

Capsules

Formula	Quantity per capsule in mg. A	B	C
Active ingredient (-) Amlodipine	0.5	2.5	5.0
Lactose	83.5	81.5	79.0
Corn Starch	15.0	15.0	15.0
Magnesium Stearate	1.0	1.0	1.0
Compression Weight	100.0	100.0	100.0

The active ingredient, (-) amlodipine, lactose, and corn starch are blended until uniform; then the magnesium stearate is blended into the resulting powder. The resulting mixture is encapsulated into suitably sized two-piece hard gelatin capsules.

Example 8

Oral Formulation

Tablets

Formula	Quantity per Tablet in Gm. A	B	C
Active ingredient, (-) amlodipine	0.5	2.5	5.0
lactose BP	183.0	181.0	178.5
starch BP	15.0	15.0	15.0
Pregelatinized Maize Starch BP			
magnesium stearate	1.5	1.5	1.5
Compression weight	200.0	200.0	200.0

The active ingredient, (-) amlodipine, is sieved through a suitable sieve and blended with lactose, starch, and pregelatinized maize starch. Suitable volumes of purified water are added and the powders are granulated. After drying, the granules are screened and blended with the magnesium stearate. The granules are then compressed into tablets using 7 mm diamter of punches.

Tablets of other strengths may be prepared by altering the ratio of active ingredient to lactose or the compression weight and using punches to suit.

What is claimed is:

1. A method of eliciting an antihypertensive effect in a human, which comprises administering to a human in need thereof a therapeutically effectiv amount of (-) amlodipine, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate hypertension.

2. The method f claim 1 wherein (-) amlodipine is administered by intravenous infusion, by transdermal delivery, or orally as a tablet or a capsule.

3. The method of claim 2 wherein the amount administered is from about 0.01 mg to about 100.0 mg daily.

4. The method of claim 3 wherein the amount administered is from about 0.5 mg to about 20 mg.

5. The method of claim 4 wherein the amount administered is from about 0.5 mg to about 10.0 mg.

6. The method of claim 1 wherein the amount of (-) amlodipine or a pharmaceutically acceptable salt thereof is greater than approximately 90% by weight of the total amount of amlodipine.

7. The method of claim 1 wherein the amount of (-) amlodipine or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, is administered together with a pharmaceutically acceptable carrier.

8. The method according to claims 2, 3, 4, 5, or 6, wherein (-) amlodipine is administered as its besylate salt.

9. A method of treating angina in a human, which comprises administering to a human in need thereof a therapeutically effective amount of (-) amlodipine, or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, said amount being sufficient to alleviate angina.

10. The method of claim 9 wherein (-) amlodipine is administered by intravenous infusion, by transdermal delivery, or orally as a tablet or a capsule.

11. The method of claim 10 wherein the amount administered is from about 0.01 mg to about 100.0 mg.

12. The method of claim 11 wherein the amount administered is from about 0.5 mg to about 20.0 mg.

13. The method of claim 12 wherein the amount administered is from about 0.5 mg to about 10.0 mg.

14. The method of claim 9 wherein the amount of (-) amlodipine or a pharmaceutically acceptable salt thereof is greater than approximately 90% by weight of the total amount of amlodipine.

15. The method of claim 9 wherein the amount of (-) amlodipine or a pharmaceutically acceptable salt thereof, substantially free of its (+) stereoisomer, is administered together with a pharmaceutically acceptable carrier.

16. The method according to claims 10, 11, 12, 13 or 14 wherein (-) amlodipine besylate is administered.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,057,344
DATED : May 2, 2000
INVENTOR(S) : James W. Young

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 1,
Line 61, replace "Davison" with -- Campbell --.

Column 9,
Line 1, delete "(-)".
Line 1, replace "isomer" with -- isomers --.
Line 65, delete "(-)"

Column 10,
Line 17, replace "(-)" with -- optically pure --.
Line 11, delete "(+)".

Signed and Sealed this

Twelfth Day of March, 2002

Attest:

Attesting Officer

JAMES E. ROGAN
Director of the United States Patent and Trademark Office